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HISTOMONIASIS IN CHICKENS: AGE OF GREATEST SUSCEPTIBILITY AND PATHOGENICITY STUDIES

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REPORTS from the field and from diagnostic laboratories have suggested an increase in the incidence of histomoniasis in chickens. This may constitute a threat to the concentrated broiler industry of the Southeast. Experimental studies were carried out to determine the age of greatest susceptibility in growing chicks. The results are herein correlated with a review of diagnostic reports from different parts of the United States on the incidence of this disease in chickens and the ages of greatest susceptibility.

INCIDENCE REPORTS OF HISTOMONIASIS IN CHICKENS

Reports from a number of diagnostic laboratories have not been evaluated. New York State records are most complete, extending back to 1947.[1] Histomoniasis in chickens calculated as percentage of the total accessions was reported as follows: 1947, 3.0; 1948, 3.6; 1949, 4.0; 1950, 4.8; 1951, 5.5; 1952, 5.3; 1953, 5.4; 1954, 3.4; 1955, 5.4; 1956, 5.4; 1957, 5.5; 1958, 5.5; and 1959, 4.8%. These percentages, somewhat higher than reports from other areas, suggest a slight increase in incidence of this disease during the 13-year period. Ipson[2] summarized accession records of Dr. Salsbury's Diagnostic Laboratories, Charles City, Iowa, for birds from the Midwest area. These reports show an increase in incidence from 0.1% in 1956 and 1957 to 3.7% in 1958, and a slight decrease to 2.8% in 1959.

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In Georgia, where accessions were chiefly broiler strains of chicks, Morris[3] showed a rather constant figure (1.6% in 1957, 1.6% in 1958 and 1.2% in 1959). In a much larger number of accessions, the averages from northeastern states[4] are but slightly higher (1.9% in 1957 and 2.3% in 1958). It may be concluded that there has been a slight increase in incidence. This is most marked in reports from the Midwest area.

Analysis of some of these reports gives some indication of the age of greatest susceptibility. In the New York State area, 7% of the total histomoniasis cases were in birds 1-4 weeks old, 81% in birds 4-20 weeks old, and 12% in birds over 20 weeks of age. The disease was recorded in 10% of all the birds brought to the laboratory in the group 4-20 weeks old but in only about 1% of the birds in the younger and older groups. Combined reports from 6 northeastern states and Canada reporting by age group showed 3.3% in the 1-4 week group, 81.3% in the 1-5 months, and 15.3% in birds older than 5 months. Ipson[2] indicated that 17% of the blackhead problems were in birds 1-6 weeks old and 83% in older birds. Hymas,[5] in working out a method of screening drugs for antihistomonal activity, concluded that chickens were most susceptible at 3 weeks of age. He had evidence that younger and older birds had more resistance to the infective agent administered as lesion materials secured from turkeys 9 days after artificial infection.

MATERIALS AND METHODS

Two experiments were completed to determine susceptibility of growing birds to histomoniasis infection at different ages. White Leghorn cockerels were raised, parasite-free, in electrically heated wire-floored batteries. Some of the infections were produced by direct inoculation of *Histomonas meleagridis* lesion materials obtained from livers and ceca of infected turkeys. The organs were ground up with physiological saline using a mortar and pestle or a Waring blender. This material was squeezed through 2 layers of cheese-cloth, and the exudates were injected rectally with a calibrated pipette. Inoculation was completed within two hours of sacrifice of the turkeys. Other infections were obtained through feeding embryonated eggs of *Heterakis gallinae* originally

recovered from worm-infected hens secured at the processing plant. Eggs were counted and administered by the method of Hansen *et al.*[6] In one experiment the design included comparisons of the two routes of inoculation and ages of greatest susceptibility.

Experiment 1. Four hundred heterakis eggs were administered to each chicken. There were 20 birds in each of four groups: 1, 32, 46 and 64 days old. Twenty-six days after inoculation, all birds were sacrificed and the extent of blackhead lesions was scored as follows: 0, no lesions visible to naked eye; 1, small nodules on the cecal epithelium; 2, thickened cecal wall, cecal plugs, or small liver lesions; 3, cecal plugs and liver lesions; 4, death from extensive lesions.

The worms were collected, counted and measured by the methods reviewed by Reid *et al.*[7]

Experiment 2. Groups of 20 birds each were compared in chickens 1, 28, 56, and 84 days old. Duplicate sets of each age group made it possible to inoculate one with lesion materials, and another received 400 embryonated *H. gallinae* eggs per bird. All birds were sacrificed 14 days after inoculation, and lesion scores, worm numbers and body weight gains were recorded. Comparisons of the data among the 4 groups were made by Duncan's[8] multiple-range test or Kramer's[9] modification, depending upon whether numbers of replications were uniform or not.

Experiment 3. The pathogenicity of a combined cecal coccidia and histomonas infection was compared with single infections of these different organisms. Forty-eight birds 33 days old were inoculated in the crop with a combined infective dose of 25,000 oocysts of *E. tenella* and 560 *H. gallinae* eggs. The same heterakis egg dose was repeated on two subsequent days. Similarly, 48 birds were given a single infection of *E. tenella* in the same dose and 48 other birds were given the same heterakis inoculations without coccidiosis exposure. Unparasitized controls were included in a fourth group. Each of the four treatments was divided into subgroups of 12 birds each, and positions randomized in batteries. Mortality data were collected and surviving birds killed for lesion scoring and worm counts at the end of two weeks.

RESULTS AND DISCUSSION

Susceptibility of birds by ages. In the first experiment, lesion scores of groups averaged 1.75 (Table 1) in 32-day-old chickens, 0.90 in both the 46- and 64-day-old groups, and 1.45 in the 1-day-old group. The 32-day-old group showed a significantly higher score than any other group. Thus it may be concluded that the most susceptible birds of the 4 ages tested were one month old.

Results from the second experiment were similar to the first in that the intermediate age groups were the most susceptible. The ranking of the heterakis-egg-infected groups in order of greatest susceptibility as indicated by mean lesion scores was as follows: 28-day, 56-day, 84-day, and 1-day. These mean scores were significantly higher with the 28-day group than the other 3 groups. With lesion materials as the source of infection the ranking by age group, beginning with highest lesion score, was: 56-day, 84-day, 28-day, and 1-day. The reasons are obscure for the difference in age susceptibility when source of infection was lesion materials compared to cecal worm eggs. Field reports and this experimental work show some variation in the week of greatest susceptibility. However, there is general agreement that birds 3 to 8 weeks of age show considerably greater susceptibility to histomoniasis than older or younger birds. This information may be useful in planning management and medication programs.

Pathogenicity in uncomplicated histomiasis infections. No mortality was induced in these three or in three similar experiments not herein reported. Comparisons of morbidity are possible through a study of weight gains during the 14-day period. Inoculated controls were not significantly different from uninoculated birds in weight gains with the number of birds on experiment. It is possible to compare weight gains of birds with high and low lesion scores. In Experiment 2, the average weights in five lesion score categories were: 0, 242g; 1, 259; 2, 258; 3, 229; and 4, 171. These results show weight loss in categories 3 and 4, which reaches statistical significance in category 4 when Kramer's[9] multiple range test is used. These data show that mild lesions did not adversely affect weight gains, but that severe lesions retarded growth.

Table 1. The effect of bird age on susceptibility to induced histomoniasis

Expt. no.	Inoculum	Age at inoculation (day)	Total worms, on necropsy (no.)	Birds positive for histomoniasis (%)	Total worm/bird	Av. worm length (mm)	Mean lesion score
#1	E[1]	1	474	25	23.7A[2]	5.93A[2]	0.45A[2]
	E	32	217	95	11.1A	4.47B	1.75B
	E	46	1026	60	51.3B	7.07C	0.90A
	E	64	587	50	29.4A	7.25D	0.90A
#2	E	1	339	20	17.0A		0.25A
	E	28	1467	100	73.4B		1.63B
	E	56	2865	100	143.3C		1.30C
	E	84	2861	100	144.1C		1.20C
	L[1]	1		11			0.11A
	L	28		75			0.75B
	L	56		95			1.11C
	L	84		75			0.85BC

[1] "E" indicates that the inoculum was 400 embryonated *Heterakis gallinae* eggs per bird administered orally. "L" indicates rectal injection of liver and cecal lesion materials from blackhead-infected turkeys.

[2] Letters refer to statistical comparisons between the four age groups using Duncan or Kramer's multiple range test. Common letters within the four age groups show non-significance, while groups not having common letters show statistical significance at the 5% level.

Histomoniasis and coccidiosis. In Experiment 3, the effects of combined infection of histomoniasis and coccidiosis were compared with single infections of *E. tenella* and of histomoniasis induced by 3 feedings of heterakis eggs. The combined infection produced 12.5% mortality in 48 birds and the coccidiosis infection produced 7.3% mortality; there was no mortality in histomonas-infected or control groups. These results strongly suggest that histomoniasis may affect mortality when combined with another disease, though the mortality rate was not high enough to show statistical significance when comparisons were made between the 4 subgroups with single and double infections.

Since the mortality and morbidity reported in field outbreaks of histomoniasis have been more severe than demonstrated in the laboratory, further work will be required to see if more pathogenic strains of *H. meleagridis* are involved or if a combination of other stress factors may be responsible for this problem.

Effects of histomoniasis and coccidiosis infections on worm numbers. Both coccidiosis and histomoniasis markedly reduced the number of cecal worms present at the end of the experimental period. Among the four sub-groups which had been divided for pen replication in Experiment 3, the average number of worms per bird was 234.7, 115.0, 105.5, and 72.1 among birds infected with *Heterakis gallinae* alone, and 36.4, 28.6, 21.8, and 20.6 in those with the worm infection combined with *E. tenella.* In Experiment 1, the highest lesion score (Table 1) in Group 2 is accompanied by the lowest worm infection and shortest worm length. These results with histomoniasis confirm observations of Baker[10] and Lund[11] showing that the disease suppresses the number and development of *H. gallinae.* Worm elimination may be due to changes in the cecal mucosa induced by the protozoan infections at the time the worm parasite migrates from the lumen into the mucosal penetration phase.

SUMMARY

1. Compiled laboratory reports indicate 1.2 to 5.5% of the chickens in diagnostic laboratories show histomoniasis.

There is some evidence of slight increase in this disease in the Midwest area.

2. When histomoniasis was experimentally induced with eggs from *Heterakis gallinae*, chickens were more susceptible at 32 days than at 1 day, 46, or 64 days of age. Comparisons with field reports indicate that very young birds and birds three months of age show some natural resistance to the organism.
3. No mortality was induced in chickens experimentally infected with *Histomonas meleagridis*. Birds with marked liver or cecal involvement show significant decreases in weight gains, but mild lesions did not significantly affect weight gains. When *Eimeria tenella* was combined with *Histomonas meleagridis*, mortality was 12.5% among 48 birds compared to 6.3% with *E. tenella* infection alone.
4. Either protozoan infection markedly reduced the numbers of cecal worms.

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ATTEMPTS TO IMMUNIZE CHICKENS AND TURKEYS AGAINST NEWCASTLE DISEASE BY THE USE OF TOBACCO MOSAIC VIRUS

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In 1958, Marxer *et al.*[3] reported that 25 mg of dried tobacco mosaic virus (TMV), diluted to 0.5 ml and injected intramuscularly, protected 5 of 6 chickens against Newcastle disease (ND). One of 2 control birds died in the same experiment. Marxer[2] also stated very briefly that it appeared possible to use TMV to immunize cattle and sheep against foot and mouth disease.

This paper reports the results of 3 trials, 2 with chickens and 1 with turkeys, that failed to immunize birds against Newcastle disease with TMV.

MATERIALS AND METHODS

The challenge virus, allanto-amniotic fluid given by intramuscular injection, was the Texas GB strain of Newcastle disease virus (NDV) obtained from the University of Wisconsin Repository for NDV (Courtesy of Dr. R. P. Hanson, University of Wisconsin, Madison). The virus was titered by passage in 10-day-old chicken embryos via the allantoic route. The titer was found to be $10^{8.3}$. In all instances, undiluted virus or a 10^4 serial dilution was used. The dose was 0.5 ml per bird.

TMV purification was essentially by the method of Schlegel and Rawlins.[5] Virus samples were increased in plants of tobacco (*Nicotiana tabacum*). About 3 weeks after inoculation,

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the leaves were harvested and frozen. For purification, the leaves were macerated in a Waring Blendor with enough water to produce a thick slurry. The homogenate was then heated 15 minutes in a water-bath at 75°C, and the cellular debris removed by centrifugation for 30 minutes at 2000 G. The supernatant was ultracentrifuged 4 hours at 75,000 G, which precipitated the TMV. The virus pellet was resuspended in either water or phosphate buffer (pH 7.0), and again centrifuged for 30 minutes at 2000 G to remove any insoluble material.

Extracts from both diseased and healthy *Nicotiana tabacum* plants were obtained by macerating the tissue in a Waring Blendor, heating the homogenate 15 minutes at 75°C, and removing the cellular debris by centrifugation. Sap from diseased plants was ultracentrifuged repeatedly until it showed no TMV activity when assayed on *Nicotiana glutinosa*. The supernatant remaining after only 1 cycle of ultracentrifugation was also used for injection. This preparation showed high TMV activity when assayed. Plant extracts were maintained at the same volumes as the tobacco sap from which they were prepared.

In Trial 1 the TMV was suspended in distilled water at a concentration of about 10 mg/ml. In Trials 2 and 3, the virus preparations were diluted with phosphate buffer (pH 7.0) to the same concentration as infected tobacco sap (about 2 mg/ml.

Purified virus preparations and plant extracts were rendered sterile from bacteria or other foreign matter by filtration through fritted glass or porcelain filters, and stored frozen.

Serum antibodies were determined with the standard hemagglutination inhibition (HI) test for ND antibodies[7] and the Ouchterlony test for TMV antibodies.[6,7]

EXPERIMENTAL

Trial 1. Thirty adult turkeys in the same pen were divided into 5 groups by wing band numbers. Preinoculation and postinoculation serum samples were collected from each group and checked for TMV and NDV antibodies.

Group 1 was given 4 intravenous injections of 1 ml of

distilled water, which was the virus diluent. Groups 2, 3, 4, and 5 were given 4 intravenous injections of 1 ml of the purified TMV suspensions, respectively strains I, II, III, and IV.[4] All injections were given 5 days apart. All birds were challenged intramuscularly 1 week after the last injection with 0.5 ml of undiluted GB strain of Newcastle virus.

Pooled preinoculation serum from these turkeys was negative for ND and TMV antibodies and remained negative for ND antibodies after the 4 injections of TMV were completed. However, TMV antibodies were demonstrated by the Ouchterlony test. When these turkeys were challenged with virulent strain of NDV, all died within 72 hours, after showing uneasiness, depression, anorexia, trembling, and prostration. No respiratory symptoms were noticeable.

Trial 2. Two hundred 2-week-old White Leghorn cockerels were divided into 8 equal groups. Groups 1 through 5 were housed in a battery in the same room. Groups 6 and 7 were penned together in the same isolation room, and group 8 was penned in a separate isolation room.

Twenty per cent of the birds were bled for a pooled serum sample before inoculation. All but group 6 were challenged with 0.5 ml of GB strain of virulent NDV diluted to 10^4.

Groups 1 through 5 received a single 0.5-ml injection intramuscularly of, respectively, 1) purified TMV suspension, strain III; 2) sterile phosphate buffer solution (pH 7.0), which was the virus diluent; 3) infective clarified plant extract from TMV-infected tobacco plants; 4) virus-free plant extract from infected tobacco plants; and 5) plant extract from healthy tobacco plants.

Groups 6 and 7 were controls, group 6 for livability and group 7 for challenge with virulent GB NDV. Group 8 was vaccinated with wing-web vaccine (American Cyanamid) and kept as vaccinated controls.

The pooled preinoculation serum from 20% of the birds was negative for NDV and TMV antibodies. Three weeks after the single injection, serum was collected from 20% of each group and pooled for postinoculation tests, but each group was tested separately. When these sera were tested, no sample showed any NDV antibodies except that from group 8, which

had been vaccinated with the wing-web vaccine. This titer was greater than 1:2048. No TMV antibodies were demonstrated from any of the groups.

All birds except control group 6 were challenged 3 weeks after the injection. All birds in all challenged groups died within 72 hours except those in group 8, vaccinated with wing-web vaccine. These 25 chickens lived. A single chicken, in group 5, that had been given extract from a healthy tobacco plant survived the challenge and showed no signs of illness at any time.

Trial 3. Ten 6-week-old cockerels were given 0.75 ml of purified TMV suspension intramuscularly each week for 5 successive weeks. Thirteen others were kept as controls. Preinoculation and postinoculation pooled serums were collected. All birds were challenged with undiluted GB strain of Newcastle virus.

Two of the 10 cockerels died before challenge, 1 from being picked and 1 from acute cecal coccidiosis. The other 8 were treated with sulfamethazine for 3 days. These chickens were negative to both NDV and TMV antibodies at the beginning of the experiment. Three weeks after the last TMV injection, serum was taken from 5 birds and pooled. The sample was negative for NDV antibodies and was positive by the Ouchterlony test for TMV antibodies. All birds died within 48 hours when challenged with virulent NDV.

DISCUSSION

Neither single nor repeated injections with purified TMV or extracts of tobacco plants produced resistance to ND in 2-week-old and 6-week-old chickens or adult turkeys. All TMV-vaccinated birds died as quickly as the unvaccinated controls. In contrast to this, the 25 chickens vaccinated with wing-web Newcastle vaccine survived the same challenge given the other chickens.

It should be mentioned that the quantity of TMV used in trials 2 and 3 for each chicken was less than that used by Marxer *et al.*[3] In trial 2, the chickens were given a single 0.5-ml injection containing 1 mg of TMV; they were neither resistant to the NDV challenge nor were TMV antibodies demonstrated by the Ouchterlony test. In trial 3 the chickens were

injected with 0.75 ml of material for 5 successive weeks. Each bird received a total of 7.5 mg of TMV. TMV antibodies were demonstrated in the pooled serum from these chickens, but they were not resistant to the NDV challenge. The adult turkeys in trial 1 received a total of 40 mg of TMV. Even though TMV antibodies were demonstrated and more TMV was given than Marxer used, there was no measurable resistance to challenge.

Hemagglutination-inhibiting antibodies for ND did not develop in birds vaccinated with TMV, and single injections of TMV did not produce serum TMV-antibody titers high enough to measure, but repeated injections did.

The single chicken from group 5 that survived the virulent NDV challenge must have been naturally resistant or else still carried a high passive immunity. The latter seems improbable for a chicken 5 weeks old. His pen mates were uniformly susceptible.

Tobacco plant extracts promptly anesthetized the birds for about 30 minutes. The reaction was typical for nicotinic anesthesia. However, no birds died and all recovered satisfactorily.

SUMMARY

Two-week-old and 6-week-old chickens and adult turkeys were injected with tobacco mosaic virus (TMV) in an attempt to immunize them against Newcastle disease. A single injection containing 1.0 mg of TMV did not produce antibodies in chickens that could be measured by the Ouchterlony test. Five successive injections of chickens with a total of 7.5 mg of TMV and 4 successive injections of turkeys with a total of 40 mg of TMV produced antibody titers demonstrable by the Ouchterlony test. All birds were challenged with virulent Newcastle disease virus, and all died within 72 hours except 25 control chickens vaccinated with wing-web Newcastle disease (ND) vaccine and a single chicken in one other group. In these experiments, TMV did not induce any resistance to ND in chickens and turkeys.

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AN OSTEOLYTIC MUCOR MYCOSIS IN A PENGUIN

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Received 9 March, 1961

EIGHT weeks before dying, a female zoo penguin (*Spheniscus humboldti*) developed an unsteady gait accompanied by photophobia involving the right eye. A tentative diagnosis was made of abscess formation involving the brain or spinal cord. Treatment with antibiotics was ineffective. The bird gradually became paralyzed and unable to stand, though it continued to eat until just before death.

LABORATORY FINDINGS

Post-mortem examination revealed a large, hard, caseous, white, homogeneous, pedunculated tumor-like lesion, about the size of a small orange, embedded beneath the lungs and involving the ribs, spinal column, spinal cord, and adjacent tissues in the area of the lung (Fig. 1). There were also small nodules in a thickened left abdominal air sac and in the anterior and posterior thoracic air sacs. The right eye was about

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1½ times normal size, and a similar type of homogeneous material was found involving the posterior chamber. A skeletal preparation revealed a lysis of the bone of the vertebral column and ribs in the affected area (Fig. 2).

Sections of the tumor-like body revealed masses of contorted, branching, non-septate hyphae throughout the necrotic tissue (Fig. 3). These stained poorly with hematoxylin-eosin, but well with Periodic-acid Schiff. Cultures at 37° yielded an apparently pure growth of a light gray, fluffy fungus with sporangia typical of the Mucoraceae. Portions of the new growth and fungus cultures, packed for submission to the Mycology Section of the Provincial Laboratory of Public Health, Edmonton, were lost, precluding pathogenicity testing. No fungus could be recovered from the remaining frozen tissues six weeks later. (The vegetative hyphae of many molds will withstand freezing, but the large non-septate hyphae of the family Mucoraceae are killed by freezing.[4]) Bacteriological examination revealed a hemolytic *Streptococcus* felt to be of secondary importance. Acid-fast bacteria were not found after antiformin digestion.

Fig. 1. Caseous mass beside cavity involving spinal cord and ribs superior to lungs. Note anatomical septum bisecting trachea.

DISCUSSION

Aspergillus fumigatus, the common cause of mycotic infections in penguins,[1,2,3] was not isolated in this case. The finding of a fungus thought to be a *Mucor*, involving the vertebral column and possibly the eye of a penguin, is unusual. Hyphae, characteristic of mucor mycosis, have not been previously seen in sections of birds.[5]

SUMMARY

An osteolytic mycotic granuloma involved the ribs and

Fig. 2. Posterior view of penguin skeleton showing lysis and exostosis of ribs and spinal column.

Fig. 3. Photomicrograph. (×500) of tumor-like lesion involving spinal column of peguin depicting branching mycelia.

spinal column in the area of the lungs of a four-year old female penguin. Sections and cultures of the lesion yielded a fungus, thought to be of the family Mucoraceae.

ACKNOWLEDGMENTS

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THE HISTOPATHOLOGY OF TURKEY "X" DISEASE IN GREAT BRITAIN

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INTRODUCTION

IN THE spring of 1960 a new "disease" of turkeys was encountered in England. Between May and August at least 500 outbreaks, involving many thousands of turkeys, were diagnosed.[1] These cases were mainly confined to the South Eastern area of England.

The history, symptoms, and gross pathology of this condition were described by Stevens *et al.*[11] and Wannop.[14] It affected turkeys 2 to 20 weeks old, with the heaviest losses in poults about one month old. Mortality was high, being 100% in many cases, and the affected birds died quickly and in good condition. Nervous symptoms and coma were often a feature of the terminal stages. No recognized pathogenic microorganism or virus was isolated. All systems of management were involved and a variety of breeds and strains were affected, although some breeds were apparently more susceptible than others. By late summer the disease had subsided, and finally ceased with the end of the turkey hatching season.

In August, 1960, a meeting of veterinary research workers, investigation officers, advisors to National Compounders, and others associated with these outbreaks recommended that the condition be known as turkey "X" disease. This paper is an account of histopathologic observations made on field cases.

MATERIALS AND METHODS

Pieces of liver, kidney, and spleen were collected from turkeys immediately after death and fixed in Susa. Serial sections were stained with hematoxylin and eosin.

Blood from individual birds was collected from the wing vein, and total counts were carried out with the method of

Natt and Herrick,[9] which has been found to give the most reliable results with fowl blood.[3] Differential counts were made on blood smears stained with Leishman's stain.

HEMATOLOGY

Total erythrocyte, leucocyte, and differential leucocyte counts were made on samples taken from birds in the advanced stage of the disease. In the majority of cases the turkeys did not live long enough to permit repeated blood examinations.

In birds with "X" disease the erythrocyte count was significantly reduced, with a marked increase in leucocyte count due mainly to an increase in heterophils and monocytes (Table 1). The heterophil count was frequently four times as great as that of healthy birds, and the monocyte count up to eight times (Fig. 12). Conversely, the lymphocyte count was proportionately reduced, in some cases to a very low figure. Since, however, this blood picture has also been found to be common in turkeys suffering from certain other infectious and parasitic conditions, it cannot be considered to be entirely characteristic of "X" disease.

LIVER

The most significant pathologic changes were in the liver, though the gross appearance of this organ was not very different from normal in some acute cases.

Liver changes occurred throughout the whole organ, and only the degree of change differed in sections taken from various positions. In acute cases the affected areas showed clearly as patches of eosinophilic parenchymatous cells with homogeneous cytoplasm (Figs. 3 and 4). This is in contrast to the more basophilic and rather granular appearance of healthy turkey liver cells after fixation with Susa (Figs. 1 and 2). These areas may be confluent and involve the whole of the section, causing the obliteration of many sinusoids and loss of structure of the liver cords (Fig. 3). Patent sinusoids and most blood vessels were frequently engorged with erythrocytes. The individual parenchymatous cells and their nuclei were distinctly swollen, and in places they contained several small

Table 1. Hematology in X disease.*

Origin	Erythrocytes (X 10^6 per cu. mm	Total leucocytes (X 10^3 per cu mm)	Differential leucocyte count (X 10^3 per cu mm)					
			Lymphocytes	Heterophils	Monocytes	Basophils	Eosinophils	Immature Forms
Normal turkey poult. (Mean of 5 birds)	2.75	25.0	15.50 (62%)	7.50 (30%)	0.75 (3%)	0.50 (2%)	0.50 (2%)	—
Case 1. X disease	1.62	44.6	6.69 (15%)	31.22 (70%)	6.69 (15%)	—	—	—
Case 2. X disease	1.04	24.2	4.35 (18%)	17.90 (74%)	1.93 (8%)	—	—	—
Case 3. X disease Poult 1	2.58	47.4	10.42 (22%)	28.91 (61%)	8.05 (17%)	—	—	—
2	2.45	28.3	4.06 (14%)	15.84 (56%)	8.49 (30%)	—	—	—
3	2.37	46.2	12.93 (28%)	24.02 (52%)	9.24 (20%)	—	—	—
4	1.89	62.3	9.34 (15%)	32.39 (52%)	20.55 (33%)	—	—	—
5 on 19/5/60	2.0	54.0	10.80 (20%)	36.72 (68%)	6.48 (12%)	—	—	—
5 on 24/5/60	1.8	47.8	1.43 (3%)	45.88 (96%)	0.47 (1%)	—	—	—

* The figures in parentheses are the relative percentages.

vacuoles within their cytoplasm (Fig. 6). A few cells contained only one large vacuole, which in some cells was compressing the nucleus slightly.

Measurements across the largest diameter of such cells gave a mean diameter of 12.2 μ, compared to 11.5 μ for an average healthy liver cell (Fig. 2). The enlarged nuclei had a mean diameter of 7.3 μ, compared to 4.6 μ for healthy hepatic nuclei. Individual nuclei were found larger than this, some measuring 11.0 μ in diameter (Fig. 6). Some of these very large nuclei were irregular in shape.

The swelling of the nuclei was frequently associated with an enlargement of the nucleolus and margination of the chromatin. As a result, the large nucleolus, which stained strongly purplish-red, stood out prominently within the large clear nucleus. This, with the vacuolated and eosinophilic cytoplasm of the swollen parenchymatous cells, was the most characteristic pathological change seen in this condition.

In many areas these cells had undergone degrees of necrobiosis, and in places only a reticulum containing cellular debris was left. No lymphocytic or granulocytic reaction occurred in these areas.

Throughout the liver small areas of regeneration of liver cells could be found, frequently adjacent to a portal tract. The new cells stained basophilically and had normal-sized nuclei; many of them showed mitotic figures (Figs. 5 and 7). The walls of some of the adjacent sinusoids appeared to be more prominent (Figs. 5 and 7). In the gross state the liver at this stage was of normal color and consistency, but enlarged.

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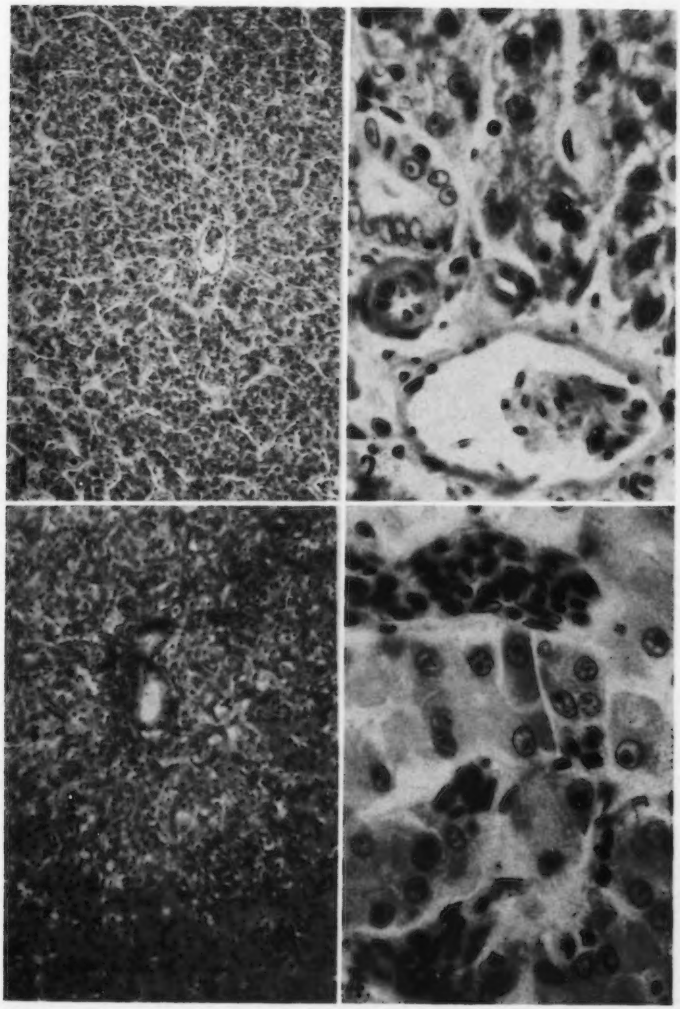
Figs. 1-4. 1) Healthy turkey liver showing lack of distinct lobulation, and the granular appearance of the parenchyma following fixation with Susa. A portal canal is shown at the center. (H and E; ×115). **2)** Higher power view of healthy turkey liver shown in Fig. 1. (H and E; ×675). **3)** Liver from a turkey in the early stages of "X" disease. Homogeneous areas with loss of cordal structure and compression of the sinusoids can be seen at the bottom left of the photograph. (×115). **4)** Liver from a turkey in the early stages of "X" disease, showing the enlarged liver cells which have eosinophilic and homogeneous cytoplasm. Some congested sinusoids are also shown. Vacuolation of the cytoplasm is commencing in a cell shown at the right of center; the nucleus of this cell shows margination of the chromatin and a prominent nucleolus. (×675).



Material from turkeys surviving longer showed more widespread regeneration of liver parenchyma, and the formation of numerous bile ductules (Fig. 8). It was difficult at this stage to be sure which cells would develop into parenchyma and which into bile ducts. The basophilic staining of these regenerating areas contrasted strikingly with the amorphous eosinophilic background of the swollen parenchyma (Fig 8). At this stage, small groups of loosely arranged lymphocytes, blast cells, and occasional plasma cells and fibroblasts could be seen in the more completely necrosed areas. In the gross state such livers were rather pale, slightly hard and swollen, with rounded edges.

The few birds that survived this condition developed pale, very hard, shrunken livers. Histologically, these livers had islets of parenchyma, some fairly normal and some consisting of swollen cells not showing the normal cordal arrangement (Fig. 9). Between these islets were areas of numerous bile ducts lying in a matrix of dead liver cells (Fig. 9), the remnants of sinusoids, and a delicate network of collagen (Fig. 10). Despite the hardness of the liver, no thick fibrous bands are present such as one might find in some cirrhotic conditions of mammals.

KIDNEY

Macroscopically, the kidneys were very enlarged, apparently from a severe congestion of the tubular sinusoids (Fig. 11).

Some degeneration of the proximal convoluted tubules was present, but this was not severe and in some sections was

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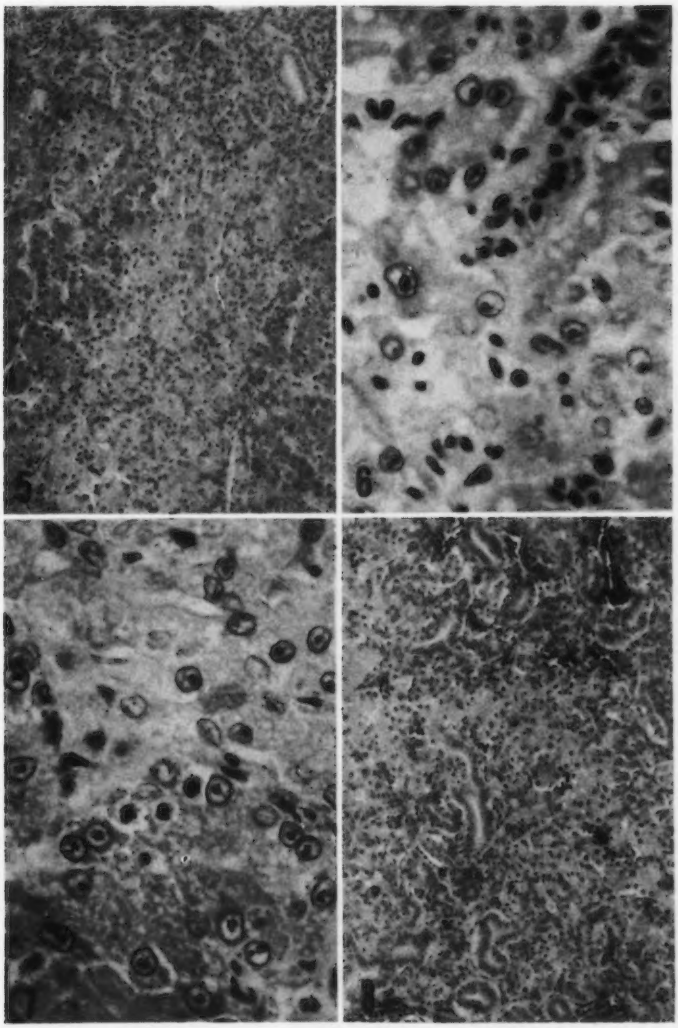
Figs. 5–8. 5) Liver from a turkey with "X" disease showing an area of degeneration at the center. Here, and in the more normal areas on each side, regeneration of liver cells has commenced where mitotic figures are present. (H and E; ×115). **6)** Turkey liver from a later stage of "X" disease than Fig. 4, showing multiple small vacuoles in the cytoplasm and many abnormal nuclei; a very large nucleus can be seen to the left of center. (H and E; ×675). **7)** Higher-power of Fig. 5, showing mitotic figures and swollen nuclei. (H and E; ×675). **8)** Turkey liver "X" disease showing the characteristic liver appearance at death. Regenerating liver cells and bile-ductule hyperplasia are shown. These stain deeply basophilically. Between are areas of pink homogeneous swollen parenchymatous cells. (H and E; ×115).



only slight. The nuclei of degenerating tubules were pyknotic and did not show the nuclear changes observed in the liver.

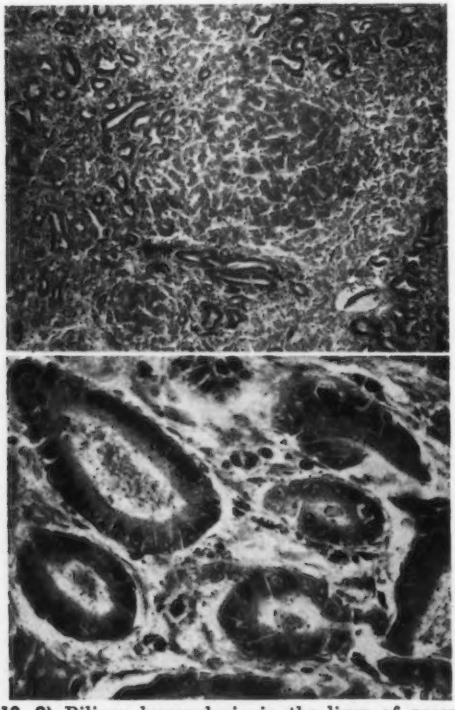
SPLEEN

The spleen showed depletion of the white pulp, and in a few cases some of the remaining lymphocyte nuclei were pyknotic. Some turkeys also showed hyperplasia of myelocytes around the sheathed arterioles.

The red pulp showed the formation of plasma cells, in some cases present in large numbers.

DISCUSSION

These pathological changes were observed in a few turkey

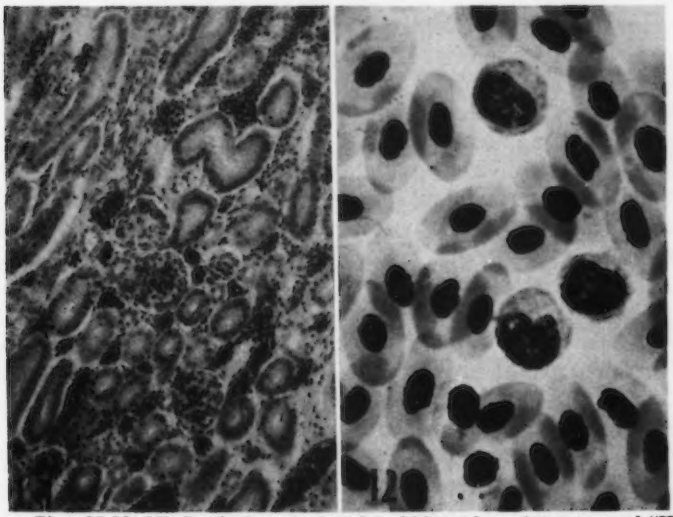


Figs. 9-10. 9) Biliary hyperplasia in the liver of recovered case of turkey "X" disease. Swollen degenerated cells are shown in center. (H and E; ×55), **10)** Higher-magnification of Fig. 9 to show the new bile ducts lying in remnants of parenchyma. Staining with green Masson showed very few collagen fibers. (H and E; × 366).

poults during 1957–59. The mortality rate was only about 15%, and cases were limited to four farms. A search of the literature has failed to reveal a similar pathological condition in turkeys.

Under intensive systems of management, poisoning of turkeys is rare and could arise only if the food contained toxic ingredients. Garner[5] makes no reference to a hepatotoxin to which turkeys may be susceptible, although he refers to several species of poisonous plants and some fungi that are known to cause liver lesions. Of these, ragwort (*Senecio* sp.) is important. On the other hand, Markson[8] was unable to produce symptoms or lesions in cockerels when a species of ragwort was mixed in the diet and fed for one month.

According to Bull,[2] the most characteristic pathological feature of poisoning by the alkaloids contained in the *Senecio* groups is megalocytosis of the liver parenchyma, associated with various degrees of replacement fibrosis, the amount de-



Figs. 11-12. 11) Cortical part of turkey kidney from late stage of "X" disease, showing severe congestion of the tubular sinusoids. No necrosis can be seen in the photograph. There are three glomeruli to the left of center. (H and E; ×115). **12)** Smear of peripheral blood; turkey "X" disease. A group of three monocytes is shown. (Leishman; ×1320).

pending on the species of animal involved. His term, megalocytosis, referred to parenchymal cells of more than double the normal diameter and with nuclei of nearly double the normal size. He did not specifically refer to the appearance of the nucleoli, but his photomicrographs showed them prominently. He described the development of focal or diffuse areas of regeneration as the intoxication progresses. An additional feature that he considered of diagnostic value was the accumulation of globules within the cytoplasm of the parenchymatous cells.

Bull[2] suggested that such changes may be common to cases of poisoning by several plants containing pyrrolizidine alkaloids. The similarity of these lesions to those described in this paper is noteworthy, though attempts failed to demonstrate the intracellular globules described by Bull.

It is dangerous to draw too strong an analogy between pathological findings in one species and those in another, but the similarity described supports the view that a poison may be involved in the etiology of this condition.

Fungal toxins have attracted more attention in recent years, and some of these can cause liver lesions in animals.[10,13] Steyn[12] refers to liver lesions in animals caused by the consumption of an alga; the toxin involved has since been shown to be an alkaloid.[7] The possibility cannot be ignored that a form of plant poison or of mycotoxicosis was involved in "X" disease, though the turkeys were not exposed to any known source of either.

Blount[1] states that he has shown that Brazilian groundnut imported in early 1960 was responsible, although the precise nature of the toxin involved has not been determined. Groundnut plants are not listed as a source of alkaloids,[6] and according to the Department of Scientific and Industrial Research[4] none have been identified in groundnut since.

Brazilian groundnut was first imported into this country at the end of 1959 and during the first half of 1960,[1] so this product could not account for the cases seen before 1960, unless groundnut from other regions may on occasion contain a similar toxic agent or agents.

SINCE THIS PAPER WAS PREPARED Siller and Ostler have described similar changes in field cases (Vet. Rec. 73, 7, 134); and a toxic substance has been extracted from Brazilian groundnut meal (Allcroft *et al.* Vet. Rec. 73, 17, 428).

SUMMARY

An account is given of histopathological changes in turkey "X" disease. These changes appear not to have been previously reported in connection with any other turkey disease. Attention is drawn to the similarity between this condition and ragwort poisoning in some mammalian species.

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OBSERVATIONS ON STRAINS OF SALMONELLA GALLINARUM APPARENTLY RESISTANT TO FURAZOLIDONE

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FOWL TYPHOID is a disease of major economic importance to the Texas poultry industry despite the fact that most commercial chickens and turkeys are hatched from pullorum-typhoid-clean stock and despite major advances in chemotherapy.

During 1960, the four Texas Poultry Disease Investigation Laboratories (at College Station, Gonzales, Center, and Stephenville) isolated *Salmonella gallinarum*, the agent of fowl typhoid, on 34 occasions from chickens and on 27 occasions from turkeys. These isolations were made from 2.62% of all chicken accessions and from 6.51% of all turkey accessions. Mortality rates in flocks of over 1000 birds were as high as 10% in a flock of 1000 chickens, and 33% in a flock of 4500 turkeys.

Furazolidone (Furoxone) has been considered the drug of choice for treating outbreaks of fowl typhoid since 1954, when Grumbles et al.[1] reported on the efficacy of the drug in treating fowl typhoid in turkeys. Other workers[2,3] have reported similar results with chickens.

S. gallinarum resistance to furazolidone has not been reported, though many organisms develop resistance to chemotherapeutic agents.

During 1960, three of the four Poultry Disease Investigation Laboratories reported one or more outbreaks of fowl typhoid in chickens and/or turkeys that did not respond to furazolidone therapy. The furazolidone levels of the medicated feeds were not always subjected to critical analysis.

To determine if furazolidone-resistant strains of *S. gallinarum* were developing, the following study was initiated.

MATERIALS AND METHODS

Strains. Isolates of *S. gallinarum* used in these experi-

ments were: 1) C-30—obtained from a flock of 6000 chickens seven months old. Losses in the flock numbered 200 birds over a 30-day period. Furazolidone was supplied to the birds during the last 2 weeks of this period at an 0.011% level in the feed—2 lb. of NF 180 (furazolidone mixture distributed by Dr. Hess and Clark, Inc.) per ton—without effecting a decrease in mortality.

2) T-419—obtained from a flock of 7500 turkeys sixteen weeks old. This flock was on range in a pen adjacent to one containing turkeys with a history of fowl typhoid and histomoniasis. As a preventive measure, the flock was medicated continuously with furazolidone at a 0.0165% level in the feed (3 lb of NF 180/ton). An outbreak of fowl typhoid occurred, and 33 birds died in 48 hours.

3) T-48—obtained from a flock of 1500 turkeys 27 weeks old. This flock was treated with furazolidone at a 0.011% level in the feed (2 lb of NF 180/ton) for two weeks before birds were submitted to the laboratory. Ten birds died in the previous 48 hours, and 50–60 birds were sick.

4) T-AN—obtained from a flock of 2700 turkeys 17 weeks old. After an initial diagnosis of fowl typhoid, this flock was given furazolidone at a 0.0192% level in the feed (3½ lb of NF 180/ton) for seven days, followed by an increase to 0.022% (4 lb NF 180/ton) for an additional nine days. The flock was treated with various levels of furazolidone (0.011% or above) for 14 more days. During this 30-day period, 500 birds died.

In vitro studies. All strains of *S. gallinarum* used in these experiments were subjected to sensitivity study *in vitro*. The second passage (lactose transfer from SS plate) of each culture was streaked on blood agar plates. Furadantin, furacin, and furoxone sensitivity disks containing 100 μg activity were placed on the plates and incubated 24 hr at 37°C.

Strains T-419 and T-AN were further studied to see if resistance to furazolidone could be induced by continuous culture passage in the presence of the drug. The procedure used is outlined in Table 1. A saturated distilled-water solution of furoxone was made by mixing an excess (50 mg) of furoxone in 250 ml of distilled water. The saturated solution (40 μg/ml) was passed through fine-porosity filter paper to re-

move the excess solute and sterilized 15 minutes in an autoclave at 17 pounds pressure.

Serial twofold dilutions of furoxone were made in sterile nutrient broth. Twenty-four-hour broth cultures of the strains were used to inoculate the furoxone dilution tubes. A tuberculin syringe with a 22-gauge needle was used to dispense one drop of broth culture into each dilution tube.

After 24 hours of incubation at 37°C, results were recorded. Turbidity was used as a measure of growth. Using the tube containing the greatest concentration of furoxone in which growth was visible, purity of culture was confirmed by appropriate microbiological techniques. The same tube was used as a source of inoculum for a second passage in furoxone dilutions. Similar techniques were used for 4 passages.

In vivo studies. Three experiments were conducted with turkeys:

Experiment 1.

Strains—C-39 and T-419 were studied in Experiment 1.

Turkeys—Beltsville White turkeys, 16 weeks old, hatched from the Veterinary Research Farm breeder supply flock, were used. Ten turkeys (6 toms and 4 hens) were assigned to each of four lots. This provided an unmedicated infected control group and a medicated infected group for each strain studied.

Inoculum—Each turkey received an intraesophageal inoculation of 1.0 ml of a saline washing from a 24-hour growth on blood agar. Strain C-39 was in the second passage in artificial media free of furoxone, and strain T-419 was in the fourth passage.

Feed—A commercial turkey grower mash was used throughout the experiment.

Medication—Furazolidone was in the form of an 11% premixture in corn meal. The drug was mixed into the feed at a concentration of 0.011% (2 lb NF 180/ton) with an electric feed mixer. Medication was started in the treatment pens at time of exposure and was continued for 21 days, at which time the experiment was terminated.

Recovery of organism—Birds that died during the experiment were cultured for *S. gallinarum* by appropriate micro-

biological techniques. Only when *S. gallinarum* was recovered were deaths attributed to fowl typhoid.

Experiment 2. This phase was conducted with the same equipment and with methods like those in Experiment 1 except as indicated.

Strains—T-419, T-48, and T-AN were used.

Turkeys—Broad-Breasted Bronze turkeys, 12 months old, commercially hatched but purchased as day-old poults and reared on the Veterinary Research Farm, were used. Since three strains of *S. gallinarum* were studied, six pens of turkeys were used.

Inoculum—Each turkey was given a 10.0-ml intraesophageal inoculation of infected liver emulsion. The emulsion was prepared by grinding infected liver tissue in equal amounts with physiological saline. The infected livers were from birds originally submitted for laboratory examination.

Medication—Treatment was started 5 days post-inoculation and continued for 23 days, at which time the experiment was terminated. Coded feed samples were subjected to critical analysis for furazolidone content as indicated in Table 2.

Experiment 3. This phase was conducted with the same equipment and with methods like those in Experiment 2 except as indicated.

Strains—T-AN was subjected to further study.

Turkeys—Beltsville White turkeys, 9 months old, hatched from the Veterinary Research Farm breeder supply flock, were used. Twenty birds (10 toms and 10 hens) were assigned to each of two lots. This provided for unmedicated infected controls, and a delayed-medication infected group. Each turkey was given a 10.0-ml intraesophageal inoculation of infected liver emulsion. Two weeks later an additional 20 birds (5 toms and 15 hens) were inoculated and placed in the medicated pen to provide a simultaneous medicated infected group.

Inoculum—The emulsion used in Experiment 3 was prepared from the liver of a bird that had died while on medication in Experiment 2.

Medication—In the delayed-treatment group, treatment was started 5 days post-inoculation and continued for 23 days.

In the simultaneous-treatment group, medication started immediately after inoculation and continued for 28 days. The feed used had been subjected to critical analysis and determined to contain 0.0111% furazolidone.

RESULTS

In vitro studies. All strains were sensitive to furadantin, furacin, and furoxone when first isolated, as evidenced by well-defined zones of growth inhibition on the sensitivity plates. Zones of inhibition approximated 8 mm, regardless of the strain examined or the disk used.

Table 1 shows the results of attempts to increase the resistance of strains T-419 and T-AN to furoxone by repeated passage in the presence of the drug. Two replicates were made for each strain.

Both strains of *S. gallinarum* were quite susceptible to the action of furoxone in the first passage. Repeated passage of T-419 failed to alter sensitivity appreciably, but T-AN developed a marked increase in resistance. The average minimum inhibiting concentration of furoxone, in μg/ml, was 0.78 in Passage 1 and 1.25 in Passage 4 when the inoculum was T-419. It was 0.24 in Passage 1 and 7.50 in Passage 4 when the T-AN strain was used.

In vivo studies. Experiment 1. Table 2 gives the experimental outline and results. Strain C-39, the chicken isolate, failed to produce morbidity or mortality in either the unmedicated or medicated groups.

Strain T-419, a turkey isolate, produced a mortality of 4 toms (40%) in the untreated group. All mortality occurred during a 4-day period 12–15 days post-inoculation. *S. gallinarum* was recovered from each dead bird. Furazolidone fed continuously at an 0.011% level prevented morbidity and mortality in the medicated group.

Experiment 2. Table 2 gives the experimental outline and results. Strain T-419 produced a mortality of 3 toms in the unmedicated group. These losses represented 30% of the pen population but 50% of the tom population in the pen. Losses occurred during a 4-day period 15–19 days post-inoculation. *S. gallinarum* was recovered from each dead bird. No hens in

Table 1. In vitro development of furoxone resistance.

Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
Drug conc. (mg/ml)	20	10	5	2.5	1.25	0.63	0.31	0.16	0.08	0.04	0.02	0 (control)
Strain T-419												
Replicate 1												
First passage	—	—	—	—	—	—	—	+[1]	+	+	+	+
Second passage	—	—	—	—	+[1]	+	+	+	+	+	+	+
Third passage	—	—	—	—	—	+[1]	+	+	+	+	+	+
Fourth passage	—	—	—	—	—	+	+	+	+	+	+	+
Replicate 2												
First passage	—	—	—	—	—	+[1]	+	+	+	+	+	+
Second passage	—	—	—	—	—	—	+[1]	+	+	+	+	+
Third passage	—	—	—	—	—	+[1]	+	+	+	+	+	+
Fourth passage	—	—	—	—	—	+	+	+	+	+	+	+
Strain T-AN												
Replicate 1												
First passage	—	—	—	—	—	—	—	—	+[1]	+	+	+
Second passage	—	—	—	—	+[1]	+	+	+	+	+	+	+
Third passage	—	—	—	+[1]	+	+	+	+	+	+	+	+
Fourth passage	—	—	—	+	+	+	+	+	+	+	+	+
Replicate 2												
First passage	—	—	—	—	—	—	—	+[1]	+	+	+	+
Second passage	—	—	—	—	—	—	+[1]	+	+	+	+	+
Third passage	—	—	—	+[1]	+	+	+	+	+	+	+	+
Fourth passage	—	—	+	+	+	+	+	+	+	+	+	+

+ positive 24-hour growth; — negative 24-hour growth.. [1] Source of inoculum for next passage.

Table 2. Typhoid mortality in experimental birds.

Lot no.	Inoculum	Medication	Typhoid mortality[a]
Experiment 1			
1	C-39	None	0/10
2	C-39	Furazolidone 0.011% at time of exposure	0/10
3	T-419	None	4/10
4	T-419	Furazolidone 0.011% at time of exposure	0/10
Experiment 2			
1	T-419	None	3/10
2	T-419	Furazolidone 0.01077%[b] started 5 days after exposure	0/10
3	T-84	None	1/10
4	T-84	Furazolidone 0.0091%[b] started 5 days after exposure	0/10
5	T-AN	None	5/10
6	T-AN	Furazolidone 0.0111%[b] started 5 days after exposure	3/10
Experiment 3			
1	T-AN	None	14/20
2	T-AN	Furazolidone 0.0111%[b] started 5 days after exposure	14/20
3	T-AN	Furazolidone 0.0111%[b] at time of exposure	8/20

[a] Deaths/totals in experiment.
[b] Furazolidone levels in the feed determined by critical laboratory analysis by Dr. Hess and Clark, Inc., Ashland, Ohio.

the unmedicated pen became visibly sick or died. Furazolidone, fed continuously starting at 5 days post-inoculation, prevented morbidity and mortality in the medicated pen.

Strain T-84 produced a mortality of one tom in the unmedicated group. This loss occurred 11 days post-inoculation, and *S. gallinarum* was isolated from the bird. Furazolidone fed continuously prevented typhoid morbidity and mortality in the medicated pen. One hen died from cannibalism. Tissues of the bird failed to yield *S. gallinarum* when cultured.

Strain T-AN produced a mortality of 5 toms in the unmedicated group. These losses represented 50% of the pen population but 83.3% of the tom population in the pen. Losses occurred during a 6-day period 12–18 days post-inoculation. *S. gallinarum* was isolated from each dead bird. Furazolidone

Table 3. Daily mortality in birds infected with strain T-AN of *Salmonella gallinarum* in Experiment 3.

Treatment	No. birds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total mortality
Infected untreated controls	20	0	0	0	0	0	0	0	2	1	2	4	1	2	1	0	1	0	0	0	0	0	14
Delayed treatment (5 days)	20	0	0	0	0	0[a]	0	1	4	3	2	3	1	0	0	0	0	0	0	0	0	0	14
Simultaneous treatment	20	0[a]	0	0	0	0	0	0	0	0	0	0	1	2	1	2	0	0	0	0	0	2	8

[a] Treatment started, furazolidone at a 0.0111% level.

fed continuously did not prevent typhoid mortality in the medicated pen. Three toms died during the post-inoculation period. These losses represented 30% of the pen population but 50% of the tom population in the pen. Losses occurred on the 17th, 19th, and 27th days post-inoculation. *S. gallinarum* was isolated from each dead bird. No hens became visibly sick or died.

Experiment 3. Tables 2 and 3 give the experimental outline and results. Strain T-AN produced a mortality of 14 birds in the unmedicated group. Mortality was equally divided between the toms and hens. Losses represented 70% of the pen population, and they occurred during a 9-day period 8–16 days post-inoculation. *S. gallinarum* was recovered from each bird that died. Furazolidone, fed continuously starting 5 days post-inoculation, did not prevent typhoid mortality. In the treated group, the losses included 7 toms and 7 hens representing 70% of the pen population. Mortality occurred during a 6-day period 7–12 days post-inoculation. *S. gallinarum* was recovered from each bird that died. Furazolidone, fed continuously starting at the time of inoculation, delayed but did not prevent typhoid mortality. Eight birds died during a 10-day period 12–21 days post-inoculation. Losses included 4 toms and 4 hens, representing 40% of the pen population. *S. gallinarum* was recovered from each bird that died.

DISCUSSION

The *in vitro* sensitivity studies showed all strains of *S. gallinarum* to be sensitive to the activity of furoxone when first isolated.

When serial passages of strain T-419 and T-AN were made in the presence of furoxone, resistance developed in the T-AN strain. Initially both strains exhibited sensitivity comparable to that of stock strains of *S. gallinarum* studied by other workers. These findings suggest that continuous or indiscriminate field use of furazolidone might allow resistant strains of *S. gallinarum* to develop.

Studies in turkeys with strains C-39 and T-84 were of no significance, because the cultures were not sufficiently pathogenic under the conditions of these experiments.

In two separate experiments, strain T-419 produced mortalities of 40 and 30% in unmedicated infected groups. Furazolidone, at an 0.011% level protected birds against losses from the T-419 strain when supplied simultaneously with inoculation or delayed 5 days. These findings correlate well with the failure of T-419 to develop resistance to furoxone *in vitro*.

In two separate experiments, furazolidone, at an 0.011% level, failed to give adequate protection against strain T-AN. This was true not only when treatment was delayed but also when treatment was started at the time of inoculation. It is postulated that the more severe losses experienced in Experiment 3 were due to increased virulence and resistance of T-AN. It had been in continuous contact with furazolidone through two turkey passages. These findings likewise correlate well with the ability of T-AN to develop *in vitro* resistance to furoxone.

All mortality in Experiments 1 and 2 occurred in male birds. If nothing else, this points up the need to use mixed populations in experiments of this nature, for if only females had been used, quite conceivably no mortality would have been produced in any group, control or medicated. On the other hand, in all likelihood, mortality would have been more severe had all-male populations been studied. The reason for the sex difference under the conditions of these experiments when controlled exposure was assured is not understood.

Differential diagnosis of poultry diseases often takes medication into consideration. Some diagnosticians hesitate to diagnose fowl typhoid when birds are known to have been on furazolidone medication. This type of thinking should be revised.

Furazolidone is still the treatment of choice for outbreaks of fowl typhoid. If experience in an individual outbreak indicates ineffectiveness, treatment should be modified accordingly.

SUMMARY

Fowl typhoid is still a problem of major importance in Texas turkeys and chickens. Three of the four Texas Poultry Disease Investigation Laboratories have reported one or more

outbreaks of fowl typhoid that failed to respond to furazolidone therapy. Four isolates of *S. gallinarum* from such outbreaks were studied in the laboratory. All were sensitive *in vitro* to furazolidone; however, one strain developed resistance when serial passages were made in the presence of the drug. The same strain displayed resistance to the activity of furazolidone in experimentally infected turkeys when the drug was supplied in the feed at a level of 0.011% (2 lb. NF 180/ton).

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CYTOPLASMIC ANTIGEN IN A LYMPHOID TUMOR OF THE CHICKEN[a]

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MALMGREN *et al.*[10] used fluorescent-antibody methods to demonstrate antigen in the nuclei of tissue-culture cells infected with polyoma virus. Malmgren *et al.*[11] demonstrated an antigen in the cytoplasm of Rous tumor cells grown in the chicken. In their work, the anti-Rous-tumor-sera used

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were prepared in chickens and turkeys by immunization with the Rous virus; the neutralizing capacity of the antiserum was measured in a tissue-culture system. Unfortunately, no *in vitro* system is yet available for determining neutralizing antibodies in chicken sera against the lymphoid tumor agent. However, it has long been known that chickens in which the transmissible lymphoid tumor has regressed are resistant to further grafts of the tumor.[15] Also there has been demonstrated[2] a cytotoxic action on the lymphoid tumor cells by serum of chickens recovered from the lymphoid tumor and subsequently given several injections of lymphoid tumor material, indicating the existence of some antibody-like substance.

Preliminary observations by Rountree[16] with the fluorescent-labeled antibody technique of Coons[3] indicated demonstrable antigen in the cytoplasm of cells of the transmissible lymphoid tumor of the chicken.[14] These observations were based on a small amount of material; further investigations are described in this report.

MATERIALS AND METHODS

The transplantable lymphoid tumor was one previously described,[14] which had been maintained in serial implant transmissions. Chickens 5–8 weeks old were inoculated in the pectoral muscles with 0.25 cc of tumor mince suspended in an equal volume of physiological saline solution. Smears were made of the tumors, which had grown to about 50 mm in diameter in seven days, and from various organs of tumor-bearing birds and of non-tumor-bearing control birds. The smears were made with a cover slip on standard glass slides, air dried, fixed in acetone for 10 minutes, dried again, and held at –10°C. The anti-tumor serum was obtained from chickens given three intramuscular inoculations, at ten-day intervals, of tumor material similar to that used for the initial inoculation after regression of their original tumors.

Serum was pooled from 12 adult chickens 10 days after the last inoculation. Gamma globulin was precipitated from the serum pool in cold alcohol by the method of Nichol and Deutsch,[12] and then conjugated with fluorescent isothiocyanate (Sylvana Chemical Co., Orange N. J., which reports the conjugate to contain 10 mg protein nitrogen per ml). It was then

adsorbed with an analytical-grade anion-exchange resin, AG2-X8 (Bio-Rod Laboratories, Richmond, California), by addition of approximately 1 g (wet-packed weight) of the resin to 5 ml of conjugate, shaken 30 min, and the resin removed by repeated centrifugation. The conjugate was then lyophilized in 1-ml amounts and kept at 4°C until use. Reconstitution of the conjugate in buffered saline, at pH 7.2, was made in a third of the original volume to intensify the specific fluorescence of the stain.

Used for the indirect method was rabbit anti-chicken gamma globulin serum conjugated with fluorescein isothiocyanate (Sylvana Chemical Co., Orange, N. J.). This conjugate was absorbed with AG 2-X8 resin and a variety of acetone-dried tissue powders from normal chickens; these powders were prepared from liver, spleen, bone marrow, and buffy coat of heparinized peripheral blood.

In the direct method, fixed, dried smears of fresh tumor were covered 30 minutes with conjugated chicken anti-lymphoid tumor gamma globulin, washed 6 minutes in three changes of phosphate-buffered saline at pH 7.2 (0.15*M* sodium chloride and 0.1*M* phosphate buffer), and mounted in glycerol with saline buffered at pH 7.2. Controls for the direct method included smears of normal chicken cells from spleen, bone marrow, and other visceral organs treated with the conjugated anti-lymphoid tumor gamma globulin. Untreated smears were also examined. Inhibition of the reaction was done with a 5% solution of unconjugated anti-lymphoid tumor gamma globulin on lymphoid tumor smears that were subsequently allowed to react with conjugated gamma globulin. This inhibiting reaction was controlled by substituting normal chicken, horse, or rabbit serum for anti-tumor gamma globulin solution.

In the indirect method the fixed smears were covered for 30 minutes at room temperature with unconjugated anti-lymphoid tumor serums, or with buffered saline containing 5% gamma globulin prepared from these serums. The rinsing procedure was carried out in the same way as in the direct method, and the smear was then covered another 30 minutes with conjugated rabbit anti-chicken gamma globulin. The smears were rinsed and mounted in the glycerin-buffered

saline mixture. Controls of tissue smears from normal chickens were treated similarly; tumor smears were treated with conjugate alone, with normal chicken serum followed by the conjugate, and with immune serum alone.

The smears were usually examined immediately after preparation, although they were sometimes kept at 4°C in a moist chamber for a few hours and then examined. The light source was a Reichert Fluorex with a 200-watt mercury-vapor lamp. A Corning #5840 ultraviolet filter was used.

RESULTS

With the indirect method, the cytoplasm of the lymphoid tumor cells usually showed a strong diffuse fluorescence. The fluorescence of the nuclei of tumor cells was much less intense (Figs. 1 and 2). These results were obtained with either anti-tumor serums or a solution of anti-tumor gamma globulin. Normal lymphoid cells fluoresced weakly or not at all (Fig. 3). The cytoplasmic granules of some granulocytes, and in some

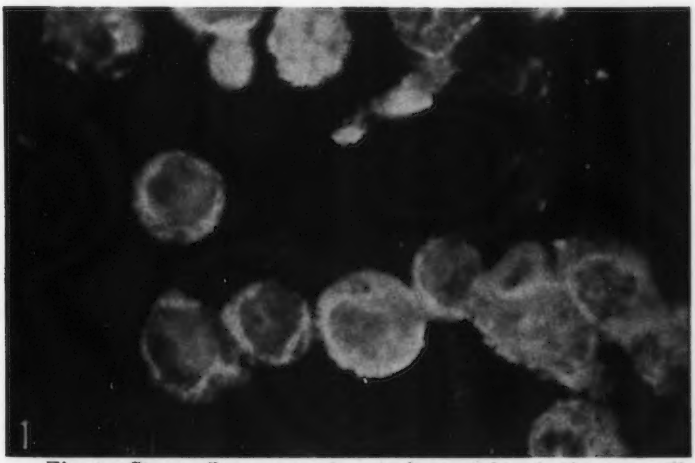

Fig. 1. Strong fluorescence in cytoplasm of lymphoid tumor cells treated by indirect method with chicken anti-tumor serum and anti-chicken rabbit gamma globulin conjugate. There is nonspecific fluorescense of nuclear and cytoplasmic margins of several erythrocytes. × 1000.

smears the cytoplasm of thrombocytes, had a strong fluorescence in both tumor-bearing and non-tumor-bearing chickens. Parenchymatous cells from the visceral organs of tumor-bearing birds (for example, cells of the liver) showed very weak fluorescence.

When normal serum was substituted for the anti-tumor serum, or only the anti-chicken conjugate was used, a weak fluorescence occurred that was similar in tumor cells and in normal lymphoid cells. Control smears not treated with conjugate showed only traces of autofluorescence. The autofluorescence was strongest in untreated smears stored several weeks at –10°C, but could be differentiated from the fluorescence of fluorescein isothiocyanate. Autofluorescence was a dull yellow, whereas the specific fluorescence of fluorescein isothiocyanate was a bright green-yellow.

Results with the direct method were similar to those with the indirect method except that the fluorescence of the cytoplasm of lymphoid tumor cells was somewhat less bright. The nonspecific fluorescence could be partially removed with tissue powders prepared from normal chicken liver or spleen.

Prior treatment of the tumor smear with unlabeled gamma globulin from immune chickens prevented the direct staining with the anti-tumor conjugate. Smears treated with a solution of normal chicken, rabbit, or horse gamma globulin and then stained with the anti-tumor conjugate still had bright

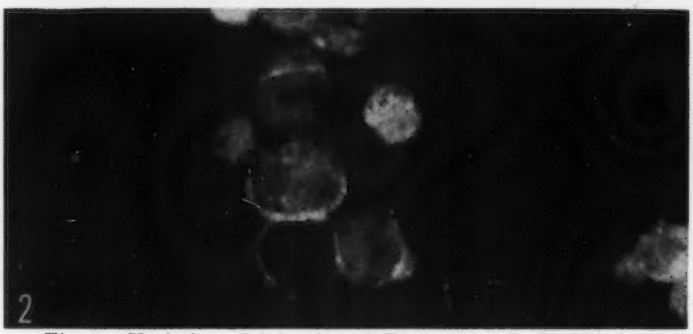

Fig. 2. Variation of intensity of fluorescence in lymphoid tumor cells treated by indirect method. × 1000.

fluorescence in the cytoplasm of most of the tumor cells. There was some decrease of the fluorescence in the nuclei of the tumor cells, and in the nonspecific fluorescence of the other types of cells.

DISCUSSION

The results confirm the observations of Rountree[5] indicating a demonstrable antigen in the cytoplasm of cells of a transmissible lymphoid tumor of the chicken. The antiserum used in its demonstration was obtained from chickens exposed to implants of tumor cells, and therefore it contained antibodies of whatever was foreign antigen in the tumor. This may have been a cellular antigen peculiar to the lymphoid tumor cell. However, the extensive adsorption procedure used in preparing the conjugate ought to have removed any cellular antigens. In addition, a cellular antigen would have appeared at the cell membrane rather than within the cytoplasm. For these reasons it is felt that the antigenic material is more probably the etiological virus of the lymphoid tumor or a type of provirus.

The presence of antigen in the cytoplasm of the lymphoid

Fig. 3. Smear of bone marrow from non-tumor-bearing chicken showing weak nonspecific fluorescence of myelocytes, normal lymphoid cells, and erythroblasts when treated with indirect method as smear illustrated in Figs. 1 and 2. × 1000.

tumor does not necessarily indicate an active virus in the cytoplasm. Electron microscopy has yielded incomplete agreement on the location of virus particles in chicken tumors. Oberling *et al.*[13] confirmed the observations of Gaylord[9] that most of the virus-like particles of Rous sarcoma were outside the cell and apparently associated with the surface of the cell membrane. The particles were found infrequently within the cytoplasm. Dmochowski *et al.*[6] have reported numerous osmophilic bodies of varying size and appearance in the tumor cells of the spleen from chickens with natural-occurring visceral lymphomatosis and experimentally induced RPL-12 lymphoid tumor. These osmophilic bodies were present in the cytoplasm and occasionally contained structures resembling virus particles, which were also scattered in the cytoplasm. These changes were found in about only one of fifty tumor cells examined. Virus-like particles were also found in the intercellular spaces. Similar structures resembling virus particles were described in the cytoplasm of cells from the spleen of chickens with erythroblastosis produced by RPL-12 material,[7] erythroblastosis caused by strain "R",[4] and granuloblastosis produced by another strain of the leukosis agent.[5] Fredrickson *et al.*[8] were unable to find virus particles in the cytoplasm of lymphoid tumor cells produced by implants, but extracellular viral particles were present and also occurred in the cytoplasm of histiocytes.

The cytoplasmic antigen in lymphoid tumor cells and in Rous sarcoma cells may represent material of a developing virus particle while it still exists inside the tumor cells. Osmophilic bodies were observed in the cytoplasm of tumor cells by Dmochowski *et al.*[6] and Bonar *et al.*[1] described a "viroplast" in the cytoplasm of myeloblasts affected with the BAI strain "A" of leukosis. Perhaps the fluorescein-labeled antibodies were attached to some similar type of provirus material in the lymphoid tumor cells of this study, even though it could not be demonstrated with the electron microscope.[8]

SUMMARY

A specific antigen was visualized by the fluorescent antibody technique in the cytoplasm of neoplastic cells from a

transmissible lymphoid tumor of the fowl. Both direct and indirect methods of staining gave a bright, specific fluorescence. The antigen may be a cellular one peculiar to the lymphoid tumor cell, or tumor virus, or provirus. The fluorescent-antibody studies are discussed in the light of electron-microscope studies of neoplastic cells from similar transmissible lymphoid tumors.

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VIRUS PARTICLES IN A LYMPHOID TUMOR OF THE CHICKEN[a]

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DESCRIPTIONS have been given of particulate virus in several transmissible fowl tumors including Rous sarcoma,[9] Fujinami myxosarcoma,[13] Murray-Begg endothelioma,[15] erythroblastosis,[4] myeloblastosis,[4] visceral lymphomatosis,[7] and a renal carcinoma.[7] In all these fowl tumors the presence of a spherical virus particle containing a dense nucleoid was reported. The particles had a diameter of about 80 mμ and an inner nucleoid 35 mμ in diameter. That particles of this size are the etiologic agent of myeloblastosis became reasonably assured when particle counts in shadowed preparations of plasma from chickens with myeloblastosis were correlated with the infectivity of these plasma samples.[8] In another study the relative infectivity of extracts of Rous sarcoma tumors was correlated with the number of particles seen in electron micrographs of sections of each tumor.[11] Similar particles, however, have been found in embryonic splenic tissue, and in fibroblast cultures from whole chicken embryos.[2] The embryos were from eggs laid by apparently normal hens. In a study of 12 embryos and chicks, all of which appeared grossly normal, five were found to have particles in their tissues.[16] The particles of virus found in the nuclei, and occasionally cytoplasm, of cultures of chicken liver cells infected with material from leukosis virus of strain RPL 12 have been shown to be a different virus from those found in other tumors of the chicken.[1] It is regarded as a contaminant GAL virus the size of which is only slightly larger than fowl tumor viruses, but its growth and crystalline

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arrangement in the nucleus are distinctive. The only differences in the ultrastructure of normal cells and tumor cells of the fowl are "gray bodies" in malignant myeloblasts,[5] and "membranous structures" described in malignant erythroblasts.[7]

This study was carried out in an effort to examine the virus particles in another fowl tumor, Olson's transmissible lymphoid tumor, and compare the ultrastructure of the malignant lymphoid cell with that of malignant myeloblasts and erythroblasts.

MATERIALS AND METHODS

The transmissible lymphoid tumor previously described[14] was propagated in this study either by inoculating 0.25 ml of minced tumor, suspended in an equal amount of physiological saline, in the pectoral muscles or by intraperitoneal inoculation of whole ascitic fluid obtained from chickens with the ascitic form of the tumor. Tumors for study were generally obtained seven days post-inoculation, and were about 30 × 20 mm in size. Various portions of the tumor were selected for study, including necrotic as well as actively proliferating areas. Sections were made of a liver and a pancreas in which metastases had occurred, and of one sample of neoplastic ascitic cells. The liver from a bird with visceral lymphomatosis was also sectioned. All tissues were fixed in Kellenberger's buffered osmium tetroxide, stained with uranyl acetate, dehydrated in methyl alcohol, and imbedded in methacrylate. Sections were placed on carbon films and examined in a Siemens Elmiskop I or II.

RESULTS

Of eleven intramuscular tumor transplants examined, six contained particles corresponding to those described for other fowl tumors (Figs. 1, 2, and 3). In the five other tumors no viral particles were seen in the sections examined. Although the particles varied in size their average diameter was 80 mμ; they had an outer double membrane and a dense inner nucleoid. In one tumor there were many such particles, but in sections of other tumors only a few were seen. The virus particles were always extracellular. They were most numerous in areas of the tumor that contained many viable-appearing tumor

cells and considerable necrotic muscle tissue. Many particles were enmeshed in this necrotic tissue. Particles were seen infrequently in areas of early infiltration, where only a few tumor cells were present and necrosis of the muscle fibers was not evident. In areas where the majority of tumor cells were necrobiotic, few particles could be seen. In the case of the ascitic tumor (Fig. 1) virus particles were adhered to the cell membrane despite considerable handling of the tumor cells during the fixation and dehydration procedures. A heterophil in the ascitic tumor contained viral particles in a phagocytic vacuole. No particles were seen in sections of the liver and pancreas in which metastatic tumor cells were present or in sections of the liver from the case of visceral lymphomatosis (Fig. 4).

The lymphoid tumor cells were round to oval, with a length of 5 to 10 μ. The cell membrane was smooth, with no pseudopodial formations although there were occasional bud-like projections (Fig. 1). Free ribosomes and endoplasmic reticulum in the form of small vesicles were scattered throughout the cytoplasm. Membranes with attached ribosomes were rare. Most of the tumor cells contained lipid bodies. The mitochondria were moderately numerous, and usually ovoid in shape with sharply defined transverse cristae. The Golgi apparatus was not prominent. The nuclei made up a large fraction of the cell and occasionally showed indentations. The nucleoli were usually large, with irregular outlines. In necrotic areas of the lymphoid tumor many necrobiotic cells were present in which swollen mitochondria filled most of the cytoplasm.

DISCUSSION

In the light microscope the cytological differences among the lymphoid tumor cells and the malignant erythroblast and myeloblast are often difficult to discern. As seen in the electron microscope, there are several structures in the ultrastructure of these cells that show certain similarities while others serve as distinguishing features for the particular cell type. All three malignant cells have large nuclei, prominent nucleoli, and an ergastoplasm composed chiefly of ribosome granules and small vesicles. Occasionally lipid bodies are observed in the cytoplasm. The malignant erythroblast has a perinuclear

Fig. 1. Tumor cell from the peritoneal cavity of a chicken with the ascitic form of the transmissible lymphoid tumor. Several virus particles (arrows) are at the cell membrane, which also shows 2 bud-like projections (b). The cytoplasm contains mitochondria (m), endoplasmic reticulum as small vesicles (v), lipid bodies (l). Extracellar debris (d) was in the ascitic fluid. The line is 1 μ long.

zone with a prominent Golgi apparatus and vacuoles containing ferratin granules.[3] The chromatin of the malignant myeloblast is usually clumped and often marginated, and "gray bodies" are present in the cytoplasm.[5] Numerous microvilli,

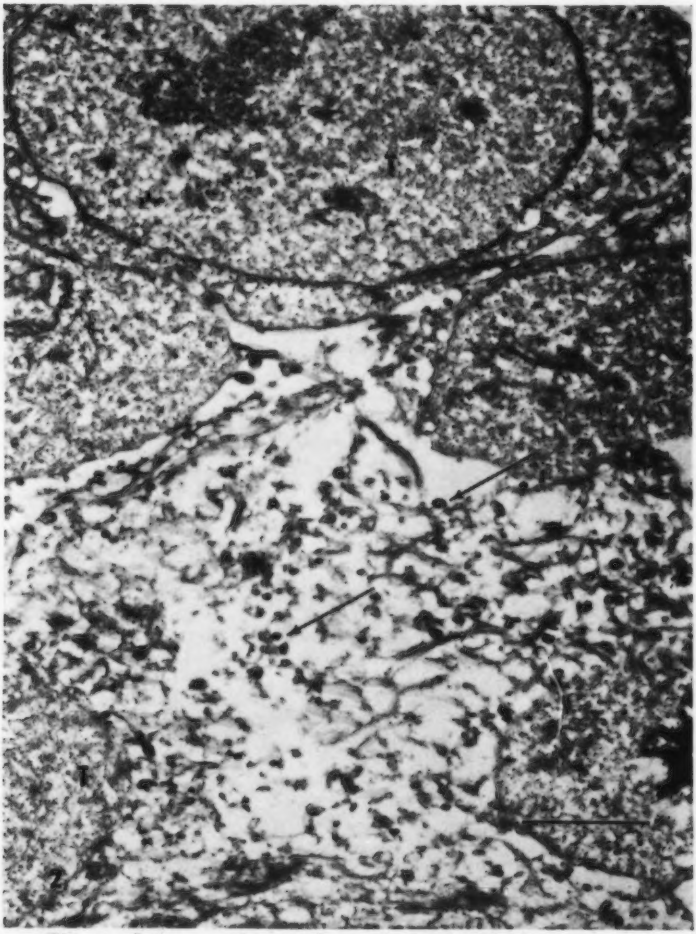

Fig. 2. Intramuscular implant of the lymphoid tumor showing tumor cells (t) and necrotic muscle fibers (n), among which many virus particles (arrows) are present. The line is 1 μ long.

processes of the cell membrane, and many cytoplasmic vacuoles have been described for malignant erythroblasts[12] and myeloblasts,[5] and may indicate active pinocytosis and phagocytosis in both types of malignant cells. The smoother cell membrane and relative lack of cytoplasmic vacuoles in malignant lymphoid cells may indicate a rate of pinocytosis and phagocytosis lower than in malignant erythroblasts and myeloblasts. Very few intracytoplasmic virus particles have been observed in any of the tumor cells of the fowl except when they have been grown in tissue cultures, and their absence in cells of the Olson lymphoid tumor was not unexpected.

Filtrates of the Olson transmissible lymphoid tumor have been shown to have oncogenic activity, and from such filtrates the RPL 12 strain of leukosis was developed.[6] It would be reasonable to assume therefore, that the particles in the tumor were tumor viruses. Some tumors appear to produce many virus particles whereas others produce very few. The ease of transplantability, as a cellular suspension, would not necessarily indicate viral content. However, in the fall of 1958, when the one tumor with many particles was observed, transmission was marked by a rapid rate of tumor growth and numerous visceral metastases, whereas several months later, when growth of the transplanted tumor was less rapid and metastases were reduced in size and frequency, the compar-

Fig. 3. A higher magnification of an area in the tumor shown in Fig. 2, showing virus particles among disintegrating myofibrils. The line is 1 μ long.

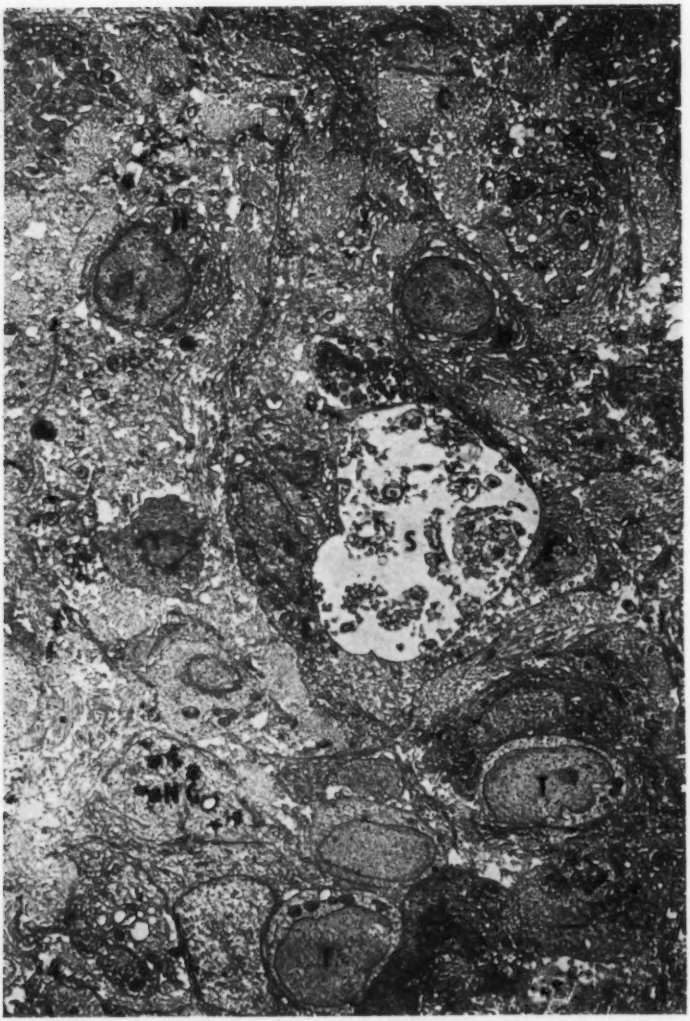

Fig. 4. Liver infiltrated with lymphoid tumor cells. A sinusoid (s), endothelial cells (e), several hepatic cells (h), and tumor cells (t) are evident. The large amount of collagen fibers (c) and necrobiotic cells (nc) indicate considerable inflammatory response of a granulomatous nature in the tumorous liver. The line is 1 μ long.

ative number of particles observed was also greatly reduced or they were totally absent. Such a correlation between tumor growth, metastases, and the number of particles seen in the tumor is inferential, and further study is necessary.

It is difficult to correlate the fluorescent-antibody studies[10] on this tumor with the electron-microscope studies since seven-day-old tumors usually exhibited intracytoplasmic fluorescence in a high percentage of the tumor cells. This is presumably a demonstration of something different from the morphologically complete virus particles since none could be found in the cytoplasm of any of the neoplastic lymphoid cells in this study. The large number of tumor cells examined in electron micrographs tends to rule out the possibility that particles were present in some of the cells but were missed. Another explanation is the existence in the cytoplasm of antigenically active provirus, which cannot be recognized in electronmicrographs. A cellular antigen, peculiar to the lymphoid tumor cell, may also have been the antigen visualized by the fluorescent-antibody technique, but this is considered unlikely.

SUMMARY

Fourteen transplants of Olson's lymphoid tumor of the fowl were sectioned and viewed with the electron microscope. Six contained extracellular virus particles morphologically similar to those described in other fowl tumors, but only one of the lymphoid tumors had large numbers of these particles. Sections of an enlarged liver from a case of visceral lymphomatosis had no particles in the sections examined.

ACKNOWLEDGMENTS

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POTENTIATION OF TERRAMYCIN II. EVALUATION OF LOW DIETARY CALCIUM IN LAYING HEN DIETS*

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CERTAIN alterations of the diet have been shown to increase the effectiveness of tetracycline antibiotics. Peterson[4] reported that the inclusion of terephthalic acid (TPA) in broiler feeds increased the antibiotic content of the blood serum by three- to fourfold. Later, Price and Zolli[5] reported that a similar increase in the serum antibiotic content could be obtained by reducing the calcium content of the diet to 0.18%. Harms and Waldroup[3] reported that decreasing the calcium content of the feed slightly below the level required by the chick gave maximum potentiation benefits as measured by the antibiotic content of the blood serum.

These experiments were made to evaluate with the laying hen the use of low-calcium diets for the potentiation of Terramycin, and to determine the effect of feeding low levels of calcium upon subsequent rate of egg production and egg shell thickness.

EXPERIMENTAL PROCEDURE

Pullets of commercial egg-production type, maintained in individual wire cages, were used in these studies. The pullets in experiment 1 had been in production for four months, and those in experiment 2 had been in production for eleven months.

The pre-experimental diet, similar to those used in commercial egg production, contained 2.30% calcium, 0.65% total phosphorus, 17% protein, and 945 Calories of productive energy per pound. This diet was modified by adjusting the level

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of ground limestone to form the eight experimental diets containing the various levels of calcium shown in Tables 1 and 2. Phosphorus content was maintained at 0.65%. Adjustments were made in the corn, soybean meal, and animal fat content of the diets in order to keep them iso-caloric and iso-nitrogenous. Each experimental diet containing 200 g of Terramycin per ton of feed was fed for five days to two replicates of five pullets each in experiment 1, and to four replicates of five pullets each in experiment 2.

At the end of the five-day experimental period, blood was obtained from each pullet by heart puncture. The Terramycin level of the blood serum was determined by the method outlined by Grove and Randall.[2] The serum Terramycin content was determined for individual birds in experiment 1; however, the blood from the five birds in each replicate was pooled for the determination in experiment 2.

Rate of egg production was calculated on a hen-day basis for the five-day potentiation period, and at five-day intervals after the birds had been returned to the pre-experimental diet. All eggs were collected on designated days for measurement of egg shell thickness, made at the equatorial plane of the egg with a Starrett ratchet micrometer.

Statements of probability in this paper are based on analysis of variance according to Snedecor,[7] with significant differences between treatment means determined by the procedure outlined by Duncan.[1]

RESULTS AND DISCUSSION

Lowering the calcium content of the diet of laying hens did not significantly change the Terramycin content of the serum (Tables 1 and 2). There was a trend for the Terramycin content of the serum to be increased when the calcium content of the diet was lowered to approx 1%; however, because of the large variation among the individual hens this difference was not statistically significant. Much of the variation among the hens may have been due to the time in the cycle of egg formation in which the blood was obtained for the antibiotic determination. It is generally known that the calcium content of the blood varies greatly during the different periods of the

Table 1. Rate of egg production, thickness of egg shells, and serum level of Terramycin from hens fed various levels of dietary calcium with 200 g of Terramycin per ton of feed for five days (experiment 1).

Dietary calcium[a] (%)	Egg production (hen day %)								Egg shell thickness (mm x 1000)						μg Terramycin/ml serum[d]
	Pre-potentiation (28 days)	During potentiation (5 days)[b]	Post-potentiation (days)						Pre-potentiation (3 days)	During potentiation (5 days)[b]	Post-potentiation (days)				
			1-5	6-10	11-15	16-20	21-25	26-30[c]			1-5	10-11	17-18[b]		
2.3	80.4	82.0^{rs}	78.6	82.6	77.6	82.6	83.0	82.6	365	401^{f}	381	373	375^{kmn}		.395
2.0	80.8	73.6^{rst}	75.0	72.6	67.4	60.0	82.6	85.0	383	434^{e}	375	388	403^{j}		.421
1.7	81.4	84.0^{r}	70.0	78.0	77.6	85.0	76.0	81.6	375	386^{fg}	347	365	363^{n}		.329
1.3	82.1	80.0^{rst}	41.6	69.2	74.2	77.6	79.2	84.0	381	373^{ghi}	335	375	370^{mn}		.395
1.0	76.8	64.0^{rstu}	48.6	74.0	71.6	78.6	64.0	70.0	393	370^{gh}	353	365	393^{jk}		.424
0.8	80.7	62.0^{stu}	41.6	69.2	74.2	77.6	79.2	67.8	391	345^{hi}	337	370	388^{jkm}		.474
0.5	80.0	60.0^{tu}	63.4	46.8	66.8	80.0	85.0	88.4	381	345^{hi}	370	368	386^{km}		.528
0.2	80.9	47.6^{u}	54.2	70.0	75.0	72.6	77.6	80.0	383	337^{i}	332	363	375^{kmn}		.556

[a] Percent calcium in diet during the five-day potentiation period. A diet containing 2.30% calcium was fed before and after the potentiation period.
[b] Means having the same superscript do not differ significantly.
[c] Means for the 26-30-day period were significantly different.
[d] Treatment means do not differ significantly.

egg-formation cycle.[6] This may account for the high level of Terramycin in the blood serum of the birds in this test since the blood samples for these determinations were obtained between 8 and 10 A.M.; therefore, they were obtained shortly after the blood attained the highest level of calcium.

Birds receiving the diets containing the lower levels of calcium during the potentiation period did not produce as many eggs as those receiving the diet containing high levels of calcium; therefore it is assumed that their calcium requirement would not be as high as for birds laying at a higher rate. From results of broiler trials[3] it appears that it might be more difficult to potentiate the antibiotic with hens laying at a low rate than with those laying at a higher rate.

Reducing the calcium level of the diet for the five-day potentiation period significantly decreased the rate of egg production (Tables 1 and 2). This adverse effect appeared to be more severe in experiment 2 than in experiment 1. The birds in experiment 2 had been in production for a longer period, and appeared to be more susceptible to the stress from the change of calcium content in the feed. When pullets in experiment 1 were returned to the feed containing 2.3% calcium, they quickly recovered. At the end of 15 days on this feed they had completely recovered as indicated by rate of egg production and shell thickness. At the end of the 20-day post-potentiation period the hens in experiment 2 had not completely recovered from the adverse effect from feeding the diets containing the low levels of calcium, as indicated by rate of egg production. The lack of recovery in experiment 2 was attributed to the fact that many of the birds were forced into a molt when the low-calcium potentiation feeds were given for five days. These results point out the necessity of considering the time that the birds have been in lay when using potentiated feeds.

Egg shell thickness decreased significantly when the diets containing the low levels of calcium were fed during the five-day potentiation period (Tables 1 and 2). However, when the birds were returned to the feed containing 2.30% calcium a recovery was observed as measured by egg shell thickness. The greatest depression was observed in experiment 2, with

Table 2. Rate of egg production, thickness of egg shells and serum level of Terramycin from hens fed various levels of dietary calcium with 200 g of Terramycin per ton of feed for five days (experiment 2).

Dietary calcium (%)[a]	Egg production (hen day %)						Egg shell thickness (mm x 1000)						μg Terramycin/ml serum[c]
	During potentiation (5 days)[b]	Post-potentiation (days)					Pre-potentiation (3 days)	During potentiation[b] (5 days)	Post-potentiation (days)				
		1-5	6-10	11-15	16-20	1-20[b]			4-5	9-10	14-15	19-20[c]	
4.1	59.0^{j}	44.5	51.2	42.9	35.5	43.6^{f}	315	353^{jk}	331	318	324	303	.368
3.5	54.0^{j}	34.5	34.7	34.2	24.0	31.9^{gh}	334	365^{k}	322	308	286	279	.311
2.9	55.0^{j}	51.3	46.0	44.7	43.0	46.3^{f}	315	334^{km}	331	322	308	299	.291
2.3	38.0^{mn}	38.0	27.0	27.0	24.0	29.0^{ghi}	313	322^{m}	312	306	290	303	.281
1.7	48.0^{k}	33.7	35.0	29.7	30.5	32.3^{gh}	319	311^{m}	301	316	300	296	.363
1.1	42.0^{m}	34.0	36.3	34.0	28.0	34.3^{g}	310	298^{n}	307	300	290	290	.438
0.5	34.0^{n}	22.8	19.9	24.3	28.5	23.8^{i}	301	270^{n}	286	307	314	306	.374
0.2	28.0^{r}	20.2	27.5	25.4	24.2	25.6^{hi}	316	260^{n}	275	289	279	286	.425

[a] Percent calcium in diet during the five-day potentiation period. A diet containing 2.30% calcium was fed before and after the potentiation period.

[b] Means having the same superscript do not differ significantly.

[c] Means were not significantly different.

the birds that had been in production for a longer period, and recovery was much slower with those birds.

SUMMARY AND CONCLUSION

Low-calcium diets were given laying hens to evaluate them for potentiating Terramycin and to study their effect on egg production and egg shell thickness. Lowering the dietary calcium level for 5 days did not significantly increase the Terramycin content of the serum.

Feeding diets containing less than 1.7% calcium lowered egg production. Birds that had been in production for 4 months recovered quickly, birds that had been in production for 11 months went into molt in some cases. More work is needed before low-calcium feeds can be recommended for prolonged periods to potentiate antibiotics for laying hens.

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THE PATHOLOGY OF PARATYPHOID INFECTION IN THE WHITE PEKIN DUCK, PARTICULARLY THE LESIONS IN THE CENTRAL NERVOUS SYSTEM

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ALTHOUGH Gaiger and Davies[4] and others[1,10] reported nervous symptoms associated with paratyphoid infection in the duck, the literature has no reference to the brain pathology. The literature contains several references to Salmonella meningitis in the human infant. Henderson[5] reported on

Fig. 1. Liver of a two-week-old duckling affected with *Salmonella typhimurium*, natural case. Necrotic foci are visible on the surface ×2.

147 human cases of which 37 were caused by *S. enteriditis*; in 13 other cases *S. typhimurium* was isolated. Levinson *et al.*[8] and Smith[11] have since added cases of Salmonella meningitis in humans. Pallaske[9] described an enzootic paratyphoid outbreak in pigeons during which a single adult bird developed torticollis. This sign was due to a purulent meningoencephalitis from which a paratyphoid organism was isolated.

During a routine examination of a two-week-old duckling, it was noted that the bird exhibited nervous symptoms similar to those associated with anatipestifer infection, i.e., locomotor incoordination and a slight head tremor. The brain was cultured on PPLO agar, and a pure culture of *S. typhimurium* was isolated from the spinal fluid. Histologic section of the brain revealed a severe heterophilic meningitis. It was decided that further studies were in order.

CLINICAL FINDINGS

Paratyphoid infection is enzootic in the ducks on Long

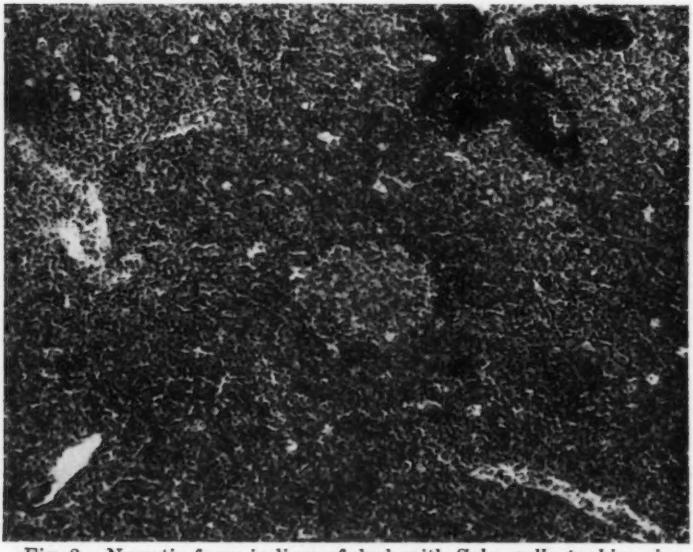


Fig. 2. Necrotic focus in liver of duck with *Salmonella typhimurium*. Upper right is an infiltration of heterophiles around a hepatic triad. ×100. H&E.

Island. The disease has been diagnosed in 463 of more than 40,000 ducks necropsied at our laboratory. *Salmonella typhimurium* has been isolated 430 times, *S. enteriditis* 9, *S. bredeney* 7, *S. anatum* 5, *S. pomona* 4, and *S. give* 2. There was one isolation each of the following: *S. newport, S. oregon, S. saintpaul, S. senftenberg, S. manhattan,* and *S. manchester.*

Clinically the outbreaks studied for brain pathology were typical of paratyphoid in Long Island ducklings. Morbidity and mortality were low, usually under 10%. Contrary to the popular conception of the disease ("Keel"), the ducklings usually died slowly, in two or three days, and became dehydrated and emaciated before death. Nephrosis was a common complication. Some gasped for air or appeared blind, with eyelids edematous and swollen shut; some trembled and occasionally developed the opisthotonus associated with duck virus hepatitis.[7] Almost always, the vents were pasted with urate.

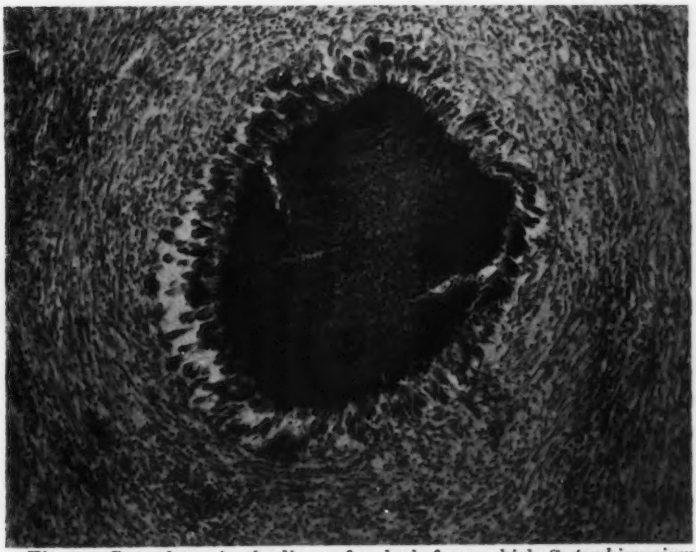


Fig. 3. Granuloma in the liver of a duck from which *S. typhimurium* was isolated. This lesion, which occurs infrequently, is probably due to a secondary complication. ×100. H&E.

PATHOLOGIC FINDINGS

Paratyphoid organisms were recovered by culture from ducklings in which the only gross lesion was a pale, slightly swollen liver; in most outbreaks, however, the classic lesions were present.

Liver. The most striking lesion in the liver was necrosis. Large foci, visible to the naked eye, were present at times (Fig. 1), but it was more common to find numerous microscopic foci throughout the parenchyma (Fig. 2). In two birds there was a ring of giant cells, composed of mononuclear phagocytes, immediately surrounding a necrotic area that contained a bright-pink, refractile, amorphous material. Peripheral to the giant cells was a zone composed of heterophiles, fibroblasts, and edema. This granulomatous lesion

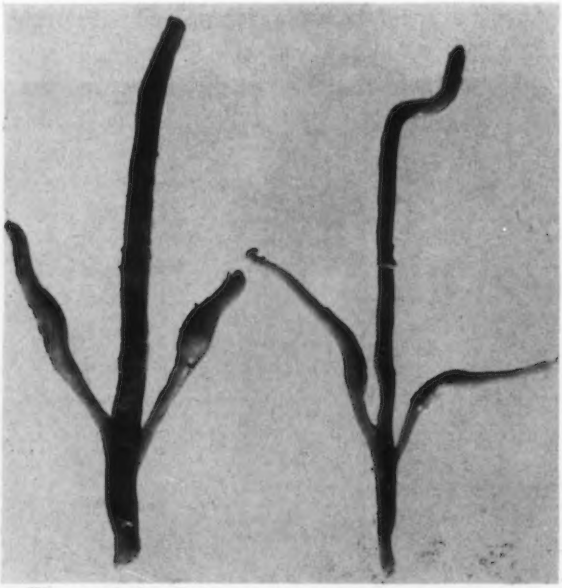


Fig. 4. Left: Ceca, rectum, and lower portion of the small intestine of a Salmonella-infected duckling. Note the enlargement of the ceca and mottling of the intestine. Right: Ceca, rectum, and part of small intestine of a normal duckling.

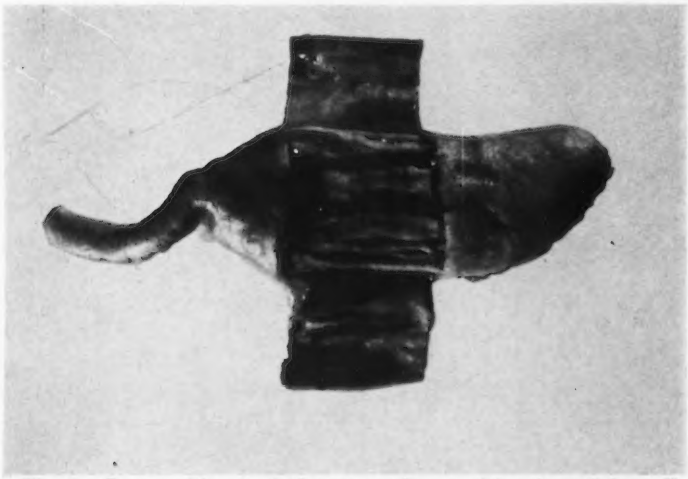


Fig. 5. Cecum with a typical caseous plug resulting from Salmonella infection.

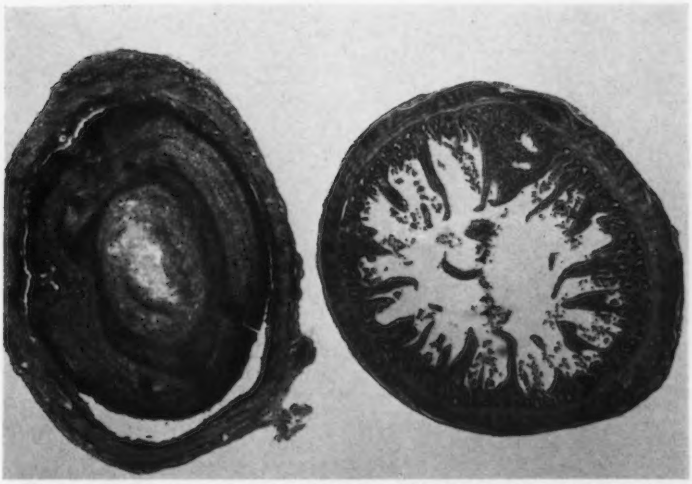


Fig. 6. Left: Cross section of cecum with Salmonella infection. Note the inflammatory exudate in the lumen and on the serosa, and the absence of villi of the mucosa. Right: Normal cecum in cross section. ×20. H&E.

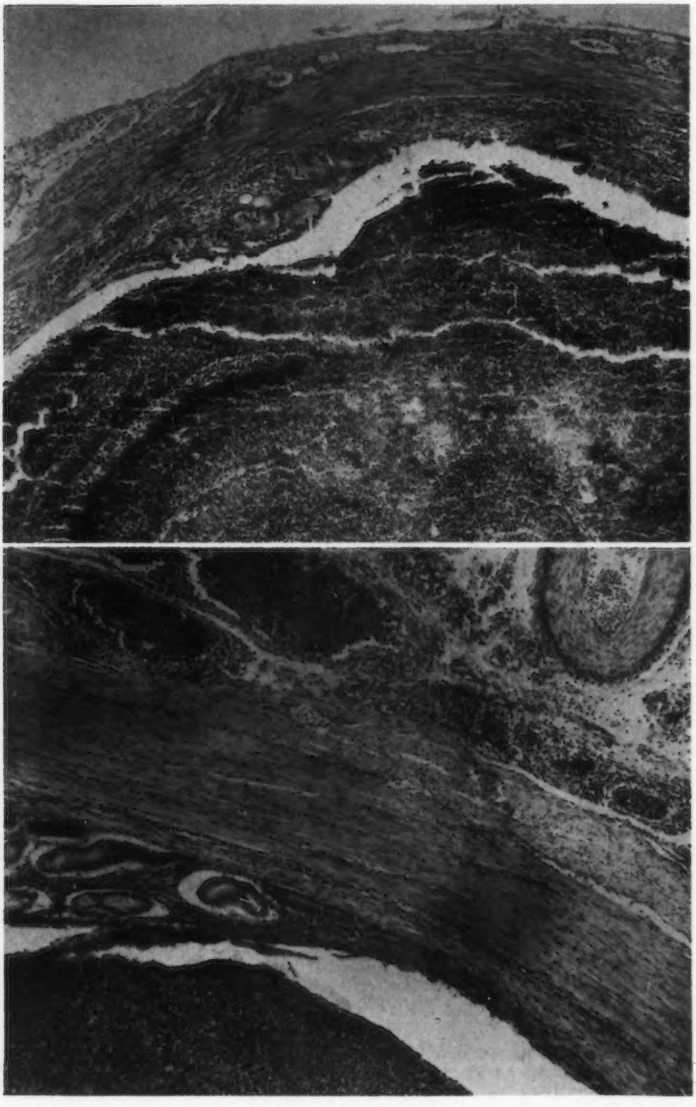


Fig. 7. Section of Salmonella-infected cecum. Note almost complete destruction of mucosa and accumulation of exudate in lumen. ×100. H&E.

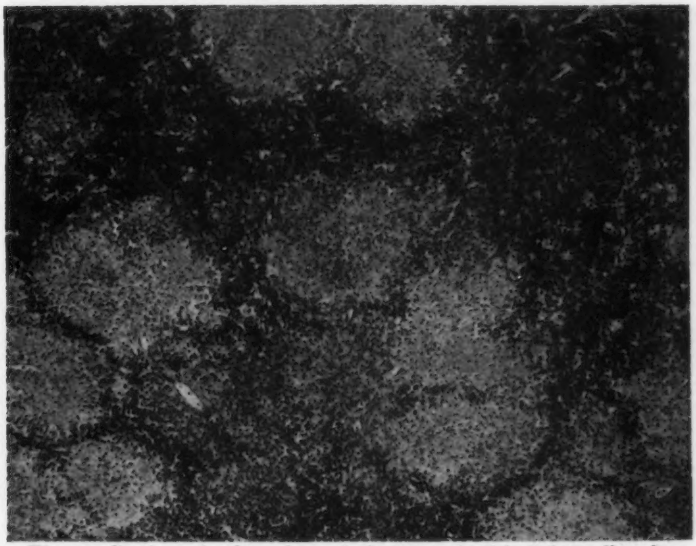


Fig. 9. Section of spleen from duck infected with Salmonella, showing necrotic foci. ×100. H&E.

Fig. 8. Cross section of rectum infected with Salmonella showing destruction of mucosa, inflammatory exudate in lumen, and peritonitis. ×100. H&E.

Fig. 11. Kidney section from a duckling with an *S. typhimurium* infection. Note casts in tubules and interstitial infiltration by inflammatory cells. ×200. H&E.

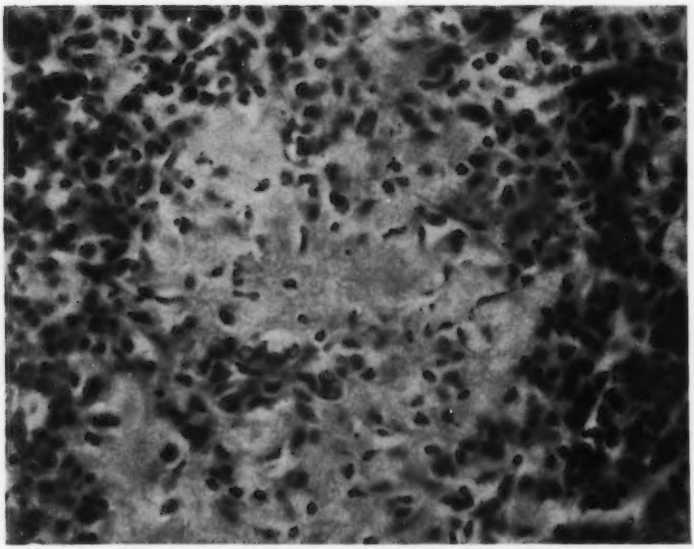
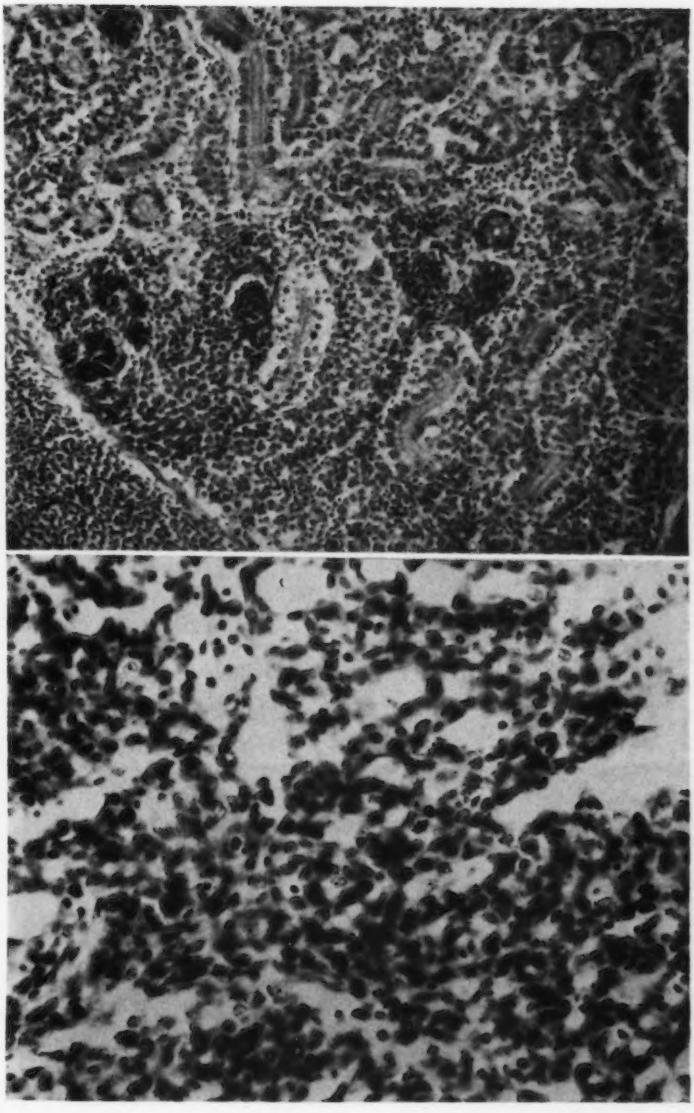


Fig. 10. High-power view of the spleen in Fig. 9. Necrotic focus is surrounded by heterophiles. ×480. H&E.

Fig. 12. Section of the lung of duckling with Salmonellosis. Lung is congested and infiltrated with heterophilic inflammatory cells. ×480. H&E.



(Fig. 3) may have been produced by a secondary infection. Similar lesions in other ducks have yielded pathogenic coliforms on culture; however, in the two birds examined, no bacteria other than *S. typhimurium* were recovered and Gridley stains did not reveal the presence of fungi.

There were numerous foci of heterophiles scattered at random throughout the liver without any relationship to lobular structure or blood vessels. There was considerable exudate, consisting chiefly of heterophiles surrounding the bile ducts (Fig. 2) and around some of the interlobular septa. The exudate around the arteries and veins was not arranged in a uniform cuff, but consisted of a single or double layer of heterophiles in some areas and deeper accumulations of cells in others. Congestion of the sinusoids was common.

Alimentary canal. Gross changes in the ceca and the rectum were common and consisted of caseous plugs in the

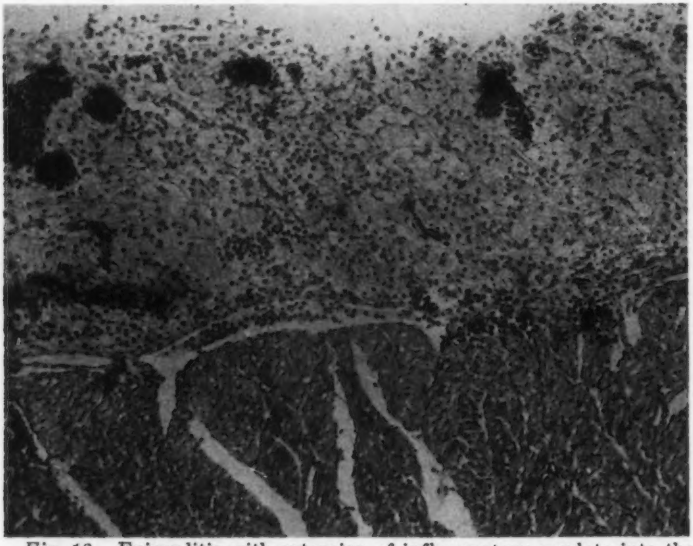


Fig. 13. Epicarditis with extension of inflammatory exudate into the myocardium in a section from a duckling with an *S. typhimurium* infection. ×100. H&E.

ceca and enlargement and mottling of the rectum and lower portion of the small intestine (Fig. 4). The cecal plugs measured as much as 1 cm in diameter and, if the birds survived, persisted for months (Fig. 5). Salmonella were isolated from these plugs in most cases. Microscopically, the lumen contained considerable exudate (Fig. 6), the mucosa was sloughed off, and in many cases only two or three necrotic glands were left (Fig. 7). The muscular and fibrous layers of the intestine were often infiltrated with heterophiles. There was at times considerable inflammatory exudate on the serosa of the intestine (Fig. 8).

Spleen. Splenomegaly up to 6 times normal size was seen. The spleen was usually pale and mottled. Microscopically there was often a diffuse infiltration of heterophiles, and occasionally focal areas of necrosis (Figs. 9 and 10).

Kidney. Grossly, the kidneys appeared pale, with an accumulation of urates in the ureters. Histologically, there

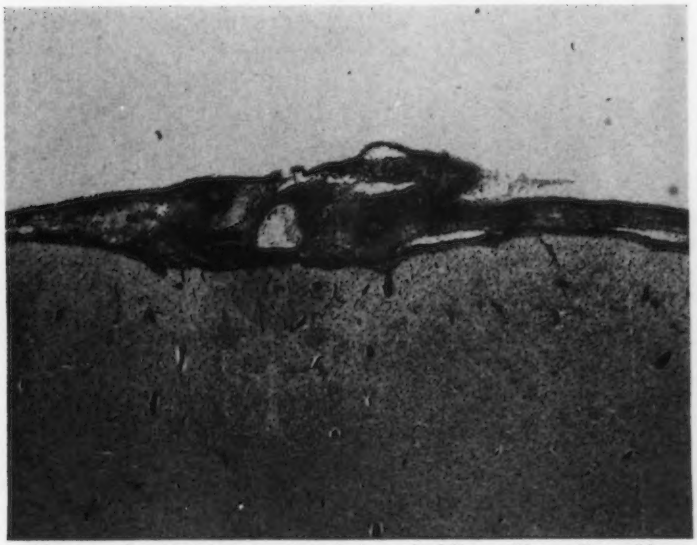


Fig. 14. Cerebral leptomeningitis in a duckling with an *S. typhimurium* infection. ×38. H&E.

was a massive interstitial infiltration of heterophiles and marked congestion of the smaller vessels. Casts were frequent in the tubules (Fig. 11).

Lungs and air sacs No gross changes were noted in the lungs. Microscopically, congestion and infiltration of the parenchyma (Fig. 12) and tunica externa and media of the pulmonary arteries with heterophiles were occasionally seen. The phlebothrombosis reported in the lungs of hamsters and mice by Innes *et al.*[6] was not a part of the pathologic picture in ducklings. A heterophilic exudate was found on the peritoneal surface of the air sacs, which macroscopically appeared normal.

Heart. A varying degree of pericarditis and epicarditis with a predominantly heterophilic exudate was noted (Fig. 13). Extension of the exudate into the myocardium was common.

Central nervous system. The lesions in the brain were

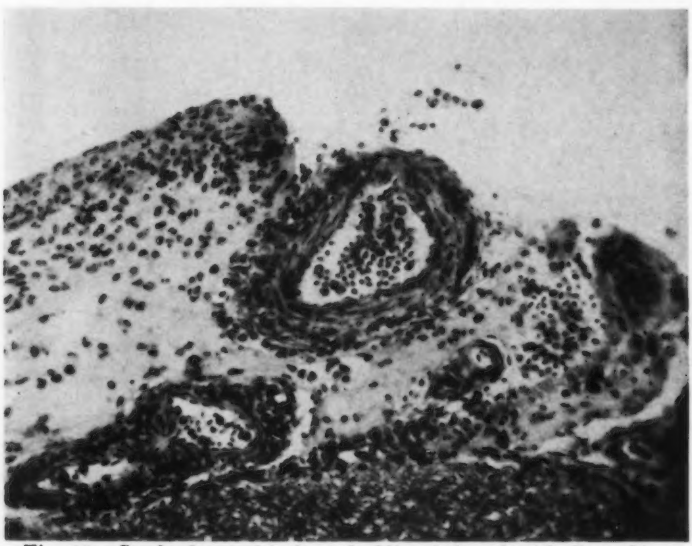


Fig. 15. Cerebral arteritis in a duckling infected with *S. typhimurium*. Heterophilic inflammation of the tunica media of an artery of the meninges. ×225. H&E.

present in ducklings that had the classic liver and intestinal lesions or no gross lesions. When the brain was involved (25 of 68 birds), the cerebral meninges appeared opaque and thickened, and seemed to be tightly drawn over the cerebral hemispheres. Histologically, an acute heterophilic leptomeningitis (Fig. 14) was present over the entire periphery of the brain. The distention of the meninges was more extensive on the dorsal portion. The inflammatory exudate was highly cellular (Fig. 14) and predominantly heterophilic. The meningeal exudate extended into the cleft between the cerebral hemispheres, causing a wide separation (Fig. 16). Inflammation of the arteries in the leptomeninges was noted (Fig. 15). The affected arteries were surrounded by a perivascular accumulation of heterophiles. There was an arteritis and periarteritis in the vessels deep in the parenchyma of

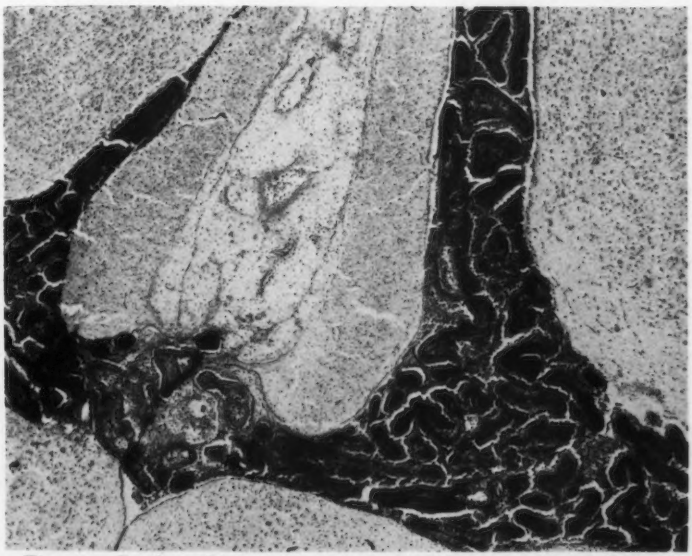


Fig. 16. Section of the cerebrum of a duckling with *S. typhimurium* infection showing meningitis in the cleft with inflammatory exudate separating the cerebral hemispheres, severe ependymitis and inflammation of the choroid plexus in lateral ventrical. ×38. H&E.

the cerebrum. Many of the heterophiles had invaded the tunica media. The lateral ventricles were greatly distended by the inflamed choroid plexi (Fig. 16). The severity and extent of the inflammation of the choroid plexus and meningitis exceeded anything previously described for ducks.[2,3] The exudate was predominantly heterophilic, with extensive accumulations in the choroidal vessels, and was free in the lateral ventricles. There was a subependimal accumulation of the glial cells in both lateral ventricles.

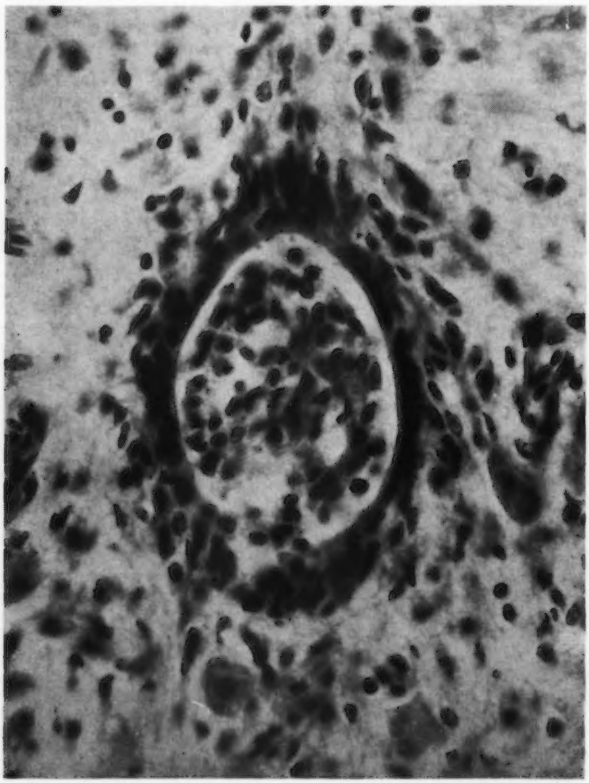


Fig. 17. Coronal section in the lumbar region of the spinal cord of a duckling with *S. typhimurium* infection. Note the heterophilic exudate in the central canal. ×480. H&E.

Perivascular cuffing was present in some vessels of the white matter of the cerebrum. No significant changes were noted in the neurons or myelin; however, in one section there was infiltration of a nerve root by mononuclear cells. Gliosis was a consistent finding.

Coronal sections of the spinal cord showed the presence of a heterophilic exudate in the central canal (Fig. 17). The leptomeninges of the spinal cord were also distended with exudate. Congestion of the vessels was noted, and there was a gliosis in the gray matter.

There was a marked optic-tract chiasm perineuritis, consisting chiefly of heterophiles. Optic neuritis was also noted (Fig. 18). This lesion undoubtedly accounts for the blindness noted under clinical signs.

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The author is indebted to Doctor P. P. Levine, New York State Veterinary College, for help in preparing the manuscript,

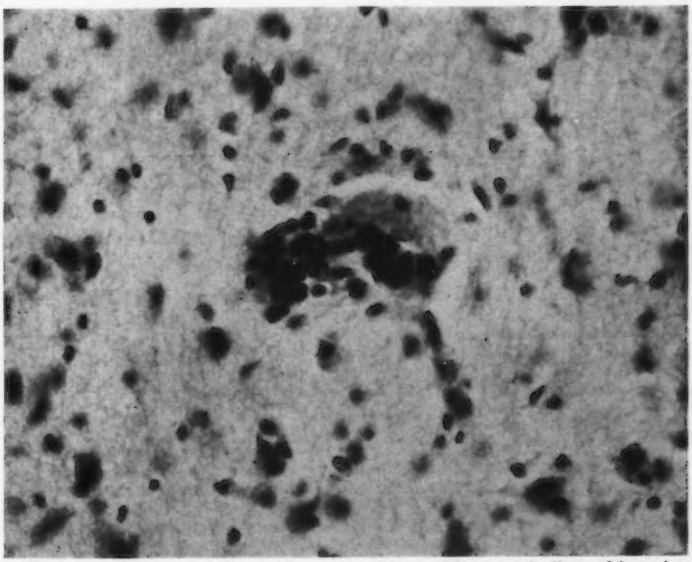

Fig. 18. Section of the optic nerve of a duckling with *S. typhimurium* infection. Note the focus of heterophilic inflammatory cells. ×480. H&E.

and to Dr. L. Z. Saunders, Smith, Kline and French Co., for assistance with interpretation of the histologic lesions.

SUMMARY

The pathology of *S. typhimurium* and *S. enteriditis* infection in the White Pekin duckling is described. Grossly, there were enlargement of the liver with or without focal necrosis, the formation of caseous plugs in the ceca, and enlargement and mottling of the rectum. Removal of the calvarium revealed thickened meninges in about one-third of the typical salmonellosis cases confirmed by bacteriologic studies.

Histologically, the lesions were inflammatory, with heterophiles predominating in the exudate. In the liver the lesions varied from a mild hepatitis to necrosis. In the spleen, kidney, and lungs there was an infiltration of heterophiles. Pericarditis, epicarditis, and myocarditis were found. The lesions in the alimentary canal consisted of degeneration and necrosis of the mucosa, with inflammatory infiltration of the muscular and fibrous layers and a peritonitis. Central nervous lesions were principally a cerebrospinal leptomeningitis and a mild encephalomyelitis. Optic neuritis and perineuritis were also noted.

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THE DEVELOPMENT OF "AIR SAC DISEASE"[a]

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The terms "air sac disease" and "chronic respiratory disease" (CRD) have been widely employed to designate a disease manifested by fibrinous pericarditis, perihepatitis, and thickening of the air sacs. It has been suggested that the disease had a very complex etiology since many agents have been isolated from accessions. Among the more important agents involved are the pathogenic *Mycoplasma* (also called pleuropneumonia-like organisms or PPLO), infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and certain bacteria most frequently identified as *Escherichia coli.*

This paper reports on methods of reproducing "air sac disease" experimentally, and makes observations on its probable natural development in the field.

MATERIALS AND METHODS

The chickens used were White Leghorns whose parents were PPLO-free and unvaccinated. They were hatched and raised in isolation, and all chickens in the experiments were housed in modified Horsfall-Bauer Units.[1,2]

The PPLO strain used is designated the "W" strain and belongs to the S-6 sterotype.[4] The inoculum was egg-propagated material in some experiments, and ground PPLO-infected air sacs in others. The NDV strain was the B_1 vaccine strain[9] propagated in embryonating eggs and preserved by holding at −56°C. Allantoic fluid containing the NDV was diluted 1:2 with tryptose broth before its use for exposure of chicks by the aerosol route.

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Table 1. Air-sac scores and weight gains following exposure to PPLO-infected birds and aerosol exposure to NDV and IBV vaccine strains.

Day of aerosol exposure		Average air-sac score	Average weight gain[a]
IBV	NDV		
1	—	1.9	216
1	1	2.0	200
14	14	6.8	172
14	—	1.3	220
1	14	6.1	199
14	1	2.2	154
—	1	1.8	164
—	14	1.8	226
—	1 and 14	1.9	169
PPLO contact control		0.9[b]	237
Uninfected control		0.0	258

[a] Weight gain in grams at 25 days of age.
[b] One bird had a score of 4.5, while 4/7 were negative.

The three IBV strains used included a strain isolated from a local laying flock with depressed egg production. The other two strains were commercial vaccines prepared from dried material according to the manufacturer's directions.

The *E. coli* strain, a pathogenic strain belonging to serotype 1-1, was used as a 24-hour tryptose broth culture containing approx. 10^8 viable bacteria per ml. Pathogenicity of coliforms refers to their ability to cause pericarditis following respiratory tract inoculation. Numbers of bacteria in the various inocula were estimated by the pour-plate method. Aerosols containing various agents were prepared with a DeVilbiss No. 40 nebulizer. Chickens were examined for lesions 5 days after coliform exposure.

The number of coliforms in feed was estimated by mixing 1 g of feed with 30 cc of brilliant green bile broth (BGBB) in a Waring blender. Numbers of coliforms were estimated by observing the growth endpoint (24 hr. at 37°C) of tenfold dilutions in BGBB.

EXPERIMENTS

Expt. 1. Groups of 8 or 10 one-day-old chicks were placed in modified Horsfall-Bauer cages. Groups of 5 similar chicks, inoculated by the air-sac route with a suspension of PPLO propagated in chicken air sac, were placed in contact with the first group. The cages were maintained at 85°F for the 1st

week, 80°F for the 2nd week, 75°F for the 3rd week, and 70°F for the 4th week. Most of the groups were exposed to NDV and/or IBV vaccines by the aerosol route according to the plan indicated in Table 1. PPLO contact without viruses and uninfected control groups were also maintained. Thirty-seven of 45 PPLO-inoculated birds died by the 9th day, and the remainder were dead by the 14th day; most had tracheal plugs. All birds were weighed at 5–7-day intervals, and all were examined for air sac lesions on the 25th day. Gross pathology on 4 groups of air sacs was scored on a scale of 0–3 (12 being the highest possible total score). These results (Table 1) indicate that, at the low brooding temperatures used, air sac lesions did develop in chicks in contact with PPLO-inoculated chicks. Exposure to vaccine viruses greatly increased the spread and severity of infection, especially when both viruses were used. The air-sac scores of individual birds were relatively uniform in all groups except the PPLO controls.

Expt. 2. This experiment actually combines the results of 3 similar experiments. Groups of 3-week-old chickens were inoculated via air sac with various combinations of PPLO and respiratory disease viruses according to the plan in Table 2. In the experiments with IBV the respiratory disease virus was inoculated at the same time at PPLO, and in the NDV experiment it was given in an aerosol 4 days later. All of the groups were exposed to aerosols containing *E. coli* 8 days after PPLO inoculation. The results (Table 2) indicate that mixed PPLO-virus infections rendered chickens very susceptible to *E. coli* pericarditis. Infection with either virus or PPLO alone was not nearly so effective.

Table 2. Incidence of pericarditis following aerosol exposure of chickens to *E. coli* following inoculation with various combinations of PPLO, NDV vaccine, IBV vaccine, and a field strain of IBV.

	IBV[a]	No IBV	IBV[b]	No IBV	NDV[b]	No NDV
PPLO	33/42	1/10	25/50	1/20	11/18	0/20
No PPLO	0/10	—	0/20		0/10	

[a] Field Strain.
[b] Vaccine Strain.

Table 3. Incidence of pericarditis following aerosol exposure of chickens to *E. coli* following air-sac inoculation with PPLO and a commercial IBV vaccine.[a]

	Expt. 1	Expt. 2
IBV[b]	0/10	0/10
IBV + PPLO[b]	0/10	0/19
IBV + PPLO [b]+ *E. coli*[c]	3/10	22/40
IBV[b] + *E. coli*[c]	0/10	0/10
PPLO[b] + *E. coli*[c]	0/10	1/10

[a] IBV and PPLO inoculated into an air sac on 1st day; *E. coli* aerosol, 6th day; necropsy 11th day.
[b] Via air sac.
[c] Aerosol exposure.

Expt. 3. Three groups of 12 chicks were exposed to NDV vaccine by the aerosol route at 10 days of age, and were later exposed to aerosols containing *E. coli.* The respective incidence of pericarditis was 4/12, 2/12, and 0/12 in chickens exposed 3½, 4½, and 6 weeks after vaccination. These results indicate that NDV vaccination alone rendered chickens susceptible to *E. coli* aerosol exposure for about 22 days. These results are similar to those reported with younger birds.[7]

Expt. 4. Groups of 3-week-old chickens were exposed to combinations of PPLO, IBV vaccine, and *E. coli* according to the plan in Table 3. The results of two experiments recorded in Table 3 indicate that combined infection with PPLO and the commercial IBV vaccine renders birds extremely susceptible to subsequent *E. coli* infection. The signs produced by all of the combinations of agents were mild.

Expt. 5. Groups of 3-week-old chickens were inoculated via air sac with both PPLO and a field strain of IBV. Groups were exposed to aerosols containing *E. coli* 4, 8, or 16 days after inoculation. Chickens were examined for pericarditis and the air sacs scored in the manner reported in expt. 1. The results (Table 4) indicate that IBV-inoculated birds were not susceptible to *E. coli* throughout the 16-day period of the experiment. The PPLO–IBV-inoculated birds were susceptible from 8 to at least 16 days after inoculation.

Expt. 6. Eighty-seven 3-week-old chickens were inoculated via air sac with both PPLO and a field strain of IBV. At various times afterward, groups of these birds were exposed to aerosols containing *E. coli.* Examination was made for

Table 4. Incidence of pericarditis and air-sac scores following aerosol exposure of chickens to *E. coli* at various times following air-sac inoculawith a commercial IBV vaccine alone or with PPLO.

	Time of *E. coli* exposure					
	4 days		8 days		16 days	
	P[a]	AS[a]	P	AS	P	AS
IBV	0/8	0.0	0/10	0.2	0/9	0.0
IBV + PPLO	1/9	4.8	3/8	3.8	3/9	2.9

[a]P=pericarditis; AS=air-sac score.

pericarditis, and the air sacs were scored in the manner reported in expt. 1. In some cases air sacs were scored for birds not exposed to aerosols containing *E. coli*. The results (Table 5) indicate that susceptibility to *E. coli* is greatest 8 days after inoculation, and lasts for at least 44 days.

Expt. 7. This experiment comprises a series of similar experiments. Groups of 3-week-old chickens were inoculated via air sac with PPLO. At various times some were exposed to NDV or field IBV by the aerosol route, and others were not exposed to viruses. Birds infected with PPLO-NDV, PPLO-IBV or PPLO were exposed to aerosols containing *E. coli* 4 days after virus exposure. The results (Table 6) indicate that chickens infected with PPLO alone are quite susceptible to *E. coli* between 16 and 41 days after inoculation. When chickens were infected with PPLO and IBV they were quite susceptible to *E. coli* between 4 and 41 days after PPLO inoculation. When chickens were infected with PPLO and NDV they were quite susceptible to *E. coli* between 4 and 8 days after PPLO inoculation and at later times were more resistant than birds with only PPLO infection.

Expt. 8. Fifty 3-week-old chickens were inoculated via air sac with PPLO and field IBV. Four days later, 10 of them

Table 5. Incidence of pericarditis and air-sac scores following exposure of chickens to aerosols containing *E. coli* at various times following PPLO-IBV air-sac inoculation.

	Day of aerosol exposure to *E. coli*							
	1	2	4	8	16	22	32	44
Pericarditis	1/8	3/7	5/8	8/8	7/11	3/6	3/5	3/4
Air-sac score	5.7	6.8	8.6	7.4	5.2	5.3	5.0	5.8
Air-sac score IBV-PPLO alone				5.4	3.5	4.6	3.8	2.6

Table 6. Incidence of pericarditis following aerosol exposure of PPLO, PPLO-IBV, or PPLO-NDV infected chickens to aerosols of *E. coli* at various times after PPLO air-sac inoculation.[a]

Day of *E. coli* exposure	NDV + PPLO	IBV + PPLO	PPLO
4	9/31	28/57	1/43
8	13/25	37/49	1/36
16	2/17	7/7	20/34
26	6/16	7/7	19/26
41	2/5	4/5	10/18
56		2/7	3/9
70		1/7	1/7

[a]IBV or NDV by aerosol exposure 4 days before *E. coli*.

were exposed to an aerosol containing *E. coli*. The other 40 were inoculated intravenously with 0.1 ml of tryptose broth containing approx. 10^7, 10^6, 10^5, or 10^4 viable *E. coli* bacteria. Groups of normal 3-week-old chickens were inoculated intravenously with 0.1 ml of tryptose broth containing 10^7, 10^6, 10^5, or 10^4 viable *E. coli* bacteria or via air sac with 10^5, 10^4 10^3, 10^2, or 10^1 viable *E. coli* bacteria. The results (Table 7) indicate that chickens were very susceptible to *E. coli* when inoculated via air sac, and rather resistant when inoculated intravenously. Respiratory-disease-infected chickens had an incidence of pericarditis similar to that of normal chickens when inoculated by the intravenous route even though they were susceptible to aerosol exposure. This suggests that the site of *E. coli* susceptibility is in the respiratory system.

In an effort to determine the source of *E. coli* infection in poultry flocks, samples of mash, feed ingredients, and litter were examined for 3 serotypes of pathogenic *E. coli*.

Of 33 samples of mash examined, 12 were positive for the 3 serotypes and 2 others contained unidentified serotypes. Even in samples containing pathogenic serotypes there were many other coliforms that could not be identified. Positive samples almost always contained more than 10^4 coliforms per gram, whereas negative samples contained no coliforms.

Thirty-five samples of feed ingredients were also examined for pathogenic serotypes. Eleven were positive, five samples contained unidentified serotypes and 19 were negative for coliforms. Among the positive ingredients were meat meal,

Table 7. Incidence of pericarditis following aerosol exposure or intravenous or air-sac inoculation of varying numbers of *E. coli* bacteria into normal or PPLO-IBV-infected birds.

Number of bacteria	Normal		PPLO-IBV infected	
	AS	IV	IV	Aerosol
10^7		5/10	4/10	5/10
10^6		3/17	0/10	
10^5	6/10	0/10	0/10	
10^4	3/10	0/10	0/10	
10^3	3/10			
10^2	3/10			
10^1	2/10			

soybean meal, corn and wheat screenings, alfalfa meal, fish meal, and cotton-seed meal.

Since commercial pelleting machines heat feed to 180–220°F, it seemed possible that pellets might be free of contamination. None of 13 samples of pellets tested contained coliforms. Of 6 samples of mash that were positive for pathogenic coliforms before pelleting, none was positive afterward.

Of 11 litter samples tested, 4 contained coliforms belonging to pathogenic serotypes, one contained an unidentified serotype, and the others were negative. The finding of negative litter samples seems surprising, though not impossible.

Rats and mice that were free from the 3 pathogens were fed contaminated feed ingredients for 1 week, and their droppings were contaminated for at least 4 months thereafter.

DISCUSSION

As a result of this work and a great deal of other recent research, a sequence of events leading to the development of coliform pericarditis can be postulated:

1) Chicks may become infected with PPLO by egg transmission[1,2,3,5,11,13] or by close contact[11] with infected birds. The lesions produced may be very mild, particularly in contact birds.[11]

2) Overcrowding, low or irregular brooding temperature, and probably other environmental factors[14] increase the transmission and severity of lesions.

3) Respiratory disease viruses such as IBV and NVD can

greatly increase the transmission and severity of PPLO infection.[1,10,11] The viruses themselves are capable of predisposing birds to subsequent coliform invasion.[6,7] Vaccine strains are almost as dangerous as field strains.

4) Of the many known serotypes of *E. coli*, only a few[6] are commonly isolated from the pericardial sacs in coliform pericarditis. These serotypes have been isolated from feed and litter samples. Rats and mice fed contaminated feed can become carriers of the pathogenic coliforms.

5) Respiratory-disease-infected chickens can be infected by small numbers of pathogenic coliforms through the respiratory tract.[6] Birds are extremely resistant to coliforms inoculated by the IV, subcutaneous, or IM routes. The susceptibility of respiratory-disease-infected chickens usually lasts several weeks.

6) Once coliform infection of the respiratory tract has occurred, the resulting disease is acute. Birds either control the infection or develop pericarditis within 2–5 days. All birds that develop pericarditis have had a bacteremia, though many birds with bacteremia fail to develop pericarditis.[8] Since several weeks are required for pericarditis lesions to heal following natural or therapeutic control of the infection, many lesions may be free of bacteria.

On the basis of this work and the work of others it seems advisable to suggest changes in the designation of the complex now called "air sac disease" or CRD. The term "chronic respiratory disease" should refer to uncomplicated PPLO infection. A similar but more severe disease caused by PPLO with IBV and or NDV should be designated "chronic respiratory disease complex." Complicated or uncomplicated coliform infection of the heart and liver should be called "coliform pericarditis."

SUMMARY

1. Chickens infected with pleuropneumonia-like organisms and infectious bronchitis virus (IBV) or Newcastle disease virus (NDV) developed pericarditis when exposed to aerosols containing pathogenic strains of *Escherichia coli*. Vaccine strains of NDV and IBV were almost as effective as field

strains. Susceptibility to *E. coli* occurred 2–44 days after inoculation. Chickens infected with PPLO alone or with viruses alone were susceptible to *E. coli* infection to a lesser degree.

2. Chickens were much more susceptible to *E. coli* when inoculated via air sac than when inoculated intravenously.

3. Pathogenic coliforms were found in feed and feed ingredients. The coliforms were destroyed during pelleting.

4. A sequence of events leading to natural *E. coli* infection was postulated.

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EXPERIMENTAL STUDIES ON CONTROL OF TRANSMISSIBLE ENTERITIS (BLUECOMB) OF TURKEYS[a]

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TRANSMISSIBLE enteritis (bluecomb) of turkeys continues to be a serious problem for turkey growers. In Ontario the greatest loss from this condition has been due to the high mortality experienced when the disease attacks poults less than six weeks old.

Peterson and Hymas,[1] Pomeroy and Sieburth,[2] Sieburth,[3] and Sieburth and Johnson[4] reported on the effect of various antibiotics in reducing the mortality caused by this disease. Their results have been varied. Studies reported by Truscott *et al.*,[5] in which various antibiotics were tested against the transmissible enteritis bacterium grown in embryonated eggs, suggested that neomycin might be of value in controlling the disease in poults.

This paper describes the results of treating groups of poults experimentally infected with transmissible enteritis. Neomycin with and without a vitamin supplement, electromycin (a penicillin - streptomycin - vitamin - salts mixture), chloramphenicol and terramycin were used in treatment and the results are compared.

MATERIALS AND METHODS

Day-old turkey poults were obtained from a hatchery thought to be free of transmissible enteritis. At no time have control poults from that source been shown to be infected, nor has there been any difficulty in producing infection in poults from that hatchery.

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[a] Presented at the 33rd annual meeting of the Northeastern Conference on Avian Diseases, June 19–21, 1961, West Virginia University, Morgantown, West Virginia.

Table 1. Experimental levels of neomycin to test effect and toxicity. Continuous treatment for seven days.

Group	Treatment	No. of birds	% mortality at 14 days
1	Infected, no drug	10	90
2	Infected + 0.35 g neomycin/gallon	10	70
3	Infected + 0.7 g neomycin/gallon	10	30
4	Infected + 1.0 g neomycin/gallon	10	90
5	Infected + 1.3 g neomycin/gallon	10	100
6	Normal controls	10	30[a]
7	0.35 g neomycin/gallon	10	0
8	0.7 g neomycin/gallon	10	100[b]
9	1.0 g neomycin/gallon	10	60
10	1.3 g neomycin/gallon	10	100

[a] Mechanical failure.
[b] Mechanical failure; no evidence of toxicity.

The groups were randomized according to weight and were weighed at three- and four-day intervals for 2–4 weeks. The birds were kept in small battery brooders maintained in isolation rooms. All medications were administered in the drinking water, which was changed daily.

Poults were infected by oral inoculation of the supernatant of an intestinal suspension prepared from birds infected with, and showing the lesions of, transmissible enteritis. This technique has been described by Truscott *et al.*[5] Percent mortality is based on the deaths occurring during the first two weeks after infection.

A preliminary experiment was conducted to determine the toxicity of neomycin in infected turkey poults. Each of the following levels of neomycin was administered to an infected and a normal group of ten day-old poults. In each case the drug was given continuously for seven days at the rate of 0, 0.35, 0.7, 1.0, and 1.3 g per gallon of drinking water. A

Table 2. Neomycin at 0.7 g/gallon for varied number of days.

Group	Treatment and days medicated	No. of birds	% mortality at 14 days
1	Infected + neomycin: 1,2,3,—6,7,8,—11,12.	22	4.5
2	Infected + neomycin: 1,2,—5,6,—9,10,—13,14.	22	9
3	Infected + neomycin: 1,2,3,—5,6,7,—9,10.	22	13.5
4	Infected, no drug	22	76.5
5	Normal controls	12	8.5

Table 3. Weight-gain studies using neomycin at 0.7 g/gallon on days 1,2,3,—6,7,8,—11,12.

Group	Treatment	No. of birds	% mortality at 14 days	Av. weight (g)	
				12 days	29 days
1	Infected, no drug	30	70	95.5	NI[a]
2	Infected + neomycin	33	9.1	115.5	427.2
3	Normal controls	30	0	174	567.6

[a] Results not included.

group of ten day-old poults were kept as normal controls.

The vitamin supplement supplied with the neomycin contained vitamins A, B_6, B_{12}, D_3, E, K, riboflavin, niacin, thiamine, folic acid, and calcium-d-pantothenate.

Terramycin was given in the drinking water at the level of 444 mg per gallon. It was given continuously for fourteen days. Chloramphenicol was tested at the same level in a similar manner.

Electromycin was given at the level of 3.0 g per gallon of drinking water. Electromycin is a formulation containing penicillin, streptomycin, riboflavin, calcium-d-pantothenate, niacin, pyridoxine, menadione sodium bisulfate, sodium ascorbate, vitamins B_{12}, A, D_3, E, and potassium chloride, calcium hydroxide, and magnesium sulfate.

RESULTS

As indicated in Table 1, a level of 0.35 g neomycin per gallon was not as effective as a level of 0.7 g per gallon, as evidenced by the greater reduction in mortality with the latter treatment. The level of 0.7 g of neomycin per gallon was decided upon for further experiments.

Table 2 shows the effect of varying the number of days of treatment with neomycin at 0.7 g per gallon. The regimen of treating on days 1, 2, 3, 6, 7, 8, 11, and 12 gave the best

Table 4. Weight-gain studies using neomycin at 0.7 g/gallon on days 1,2,3,—6,7,8,—11,12.

Group	Treatment	No. of birds	% mortality at 14 days	Av. weight (g)		
				7 days	14 days	28 days
1	Infected, no drug	26	73	52.1	NI	NI
2	Infected + neomycin	26	20	66.5	114.7	362
3	Normal controls	26	0	84.4	148	488

Table 5. A comparison of neomycin at 0.7 g/gallon on days 1,2,3,—6,7,8,—11,12, with terramycin at 444 mg/gallon given continuously for 14 days.

Group	Treatment	No. of birds	% mortality at 14 days	Av. weight (g) at 14 days
1	Infected, no drug	43	88.4	87
2	Infected + neomycin	30	6.7	145
3	Infected + terramycin	30	63.3	104.6

results. This level and schedule was used for all further trials with neomycin.

Though neomycin was quite effective in reducing mortality, the results recorded in Tables 3 and 4 show that it overcomes the debilitating effect of the disease only partially. At four weeks of age the normal controls were an average of 140.4 g (Table 3) and 126 g (Table 4) heavier than the infected neomycin-treated birds.

The results in Tables 5 and 6 show that neither terramycin nor chloramphenicol at the levels employed gave as good results as neomycin.

The results in Tables 5 and 6 show that neither terramycin nor chloramphenical at the levels employed gave as good results as neomycin.

The results of treatment with electromycin, neomycin, and neomycin plus vitamins are compared in Table 7. They indicate that there is little difference between neomycin and electromycin. The inclusion of vitamins with neomycin was associated with increased gains in one experiment and decreased gains in two experiments.

DISCUSSION AND SUMMARY

Terramycin and chloramphenicol at the levels used in this study were not as effective as neomycin in reducing mortality

Table 6. A comparison of neomycin at 0.7 g/gallon on days 1,2,3,—6,7,8,—11,12 with chloramphenicol at 444 mg/gallon given continuously for 14 days.

Group	Treatment	No. of birds	% mortality at 14 days	Av. weight (g)		
				7 days	14 days	21 days
1	Infected, no drug	25	84	52	NI	NI
2	Infected + neomycin	25	20	65.1	112.3	211.2
3	Infected+chloramphenicol	25	44	60.7	99	181.4
4	Normal controls	25	4	116.1	217	365.5

Table 7. Comparisons of neomycin with neomycin plus vitamins and with electromycin.

Group	Treatment	No. of birds	% mortality at 14 days	Av. weight (g) 7 days	14 days	21 days	28 days
Exp. 1							
1	Infected, no drug	20	65	60	NI	NI	NI
2[a]	Infected + neomycin	21	9.5	75.8	121.2	186.2	288.7
3[b]	Infected + neomycin + vitamins	20	9.5	79.7	135.2	207.1	336.3
4[c]	Infected + electromycin	21	14.3	71.7	127.6	197.5	321.8
5	Normal controls	20	0	114.4	211	318.5	503.5
Exp. 2							
1	Infected, no drug	35	89	52.8	NI	NI	NI
2[a]	Infected + neomycin	35	23	70.2	127.7	206.2	376.8
3[b]	Infected + neomycin + vitamins	35	23	68.8	112.9	193.7	326.8
4[d]	Infected + neomycin + vitamins	35	3	72.1	130.2	201.7	353
5[c]	Infected + electromycin	35	26	68.1	117.4	196.3	338.5
6	Normal controls	30	3	104.2	188.6	334.9	515.9
Exp. 3							
1	Infected, no drug	26	88.3	58.2	NI	NI	
2[a]	Infected + neomycin	26	0	70.9	126.7	201.8	
3[b]	Infected + neomycin + vitamins	26	15.4	72.9	116	188.8	
4[c]	Infected + electromycin	26	23	70	126.4	214.3	
5	Normal controls	26	3.8	106	206	358	

[a] Neomycin 0.7 g/gallon on days 1, 2, 3,—6, 7, 8,—11, 12.

[b] Vitamins A, B_6, B_{12}, D_3, E, K, riboflavin, niacin, thiamine, folic acid, calcium-d-pantothenate, administered on same days as neomycin.

[c] Electromycin, a penicillin streptomycin formulation including riboflavin, calcium-d-pantothenate, niacin, pyridoxine, menadione sodium bisulfate, sodium ascorbate, vitamins B_{12}, A, D_3, E, and potassium chloride, calcium hydroxide and magnesium sulfate administered at the level of 3.0 g/gallon on days 1, 2, 3, 4, 5,—8, 9, 10, 11, 12.

[d] Vitamins as for group [b] administered continuously for 14 days.

or in maintaining weight gains in birds infected with transmissible enteritis. Both electromycin and neomycin effectively reduced mortality and improved weight gains. A vitamin supplement with neomycin does not appear to increase weight gains or reduce mortality consistently beyond that attained with neomycin alone.

Neomycin and electromycin were effective in reducing mortality and helping maintain weight gains in diseased birds. They only partially overcame the debilitating effect of transmissible enteritis in birds to four weeks of age, as evidenced by the heavier weights attained by the normal controls. Since

facilities were not available to maintain the birds to maturity, the long-term effect of this early morbidity was not determined.

Administering neomycin at higher levels may result in toxic manifestations.

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STUDIES ON AVIAN ENCEPHALOMYELITIS. III. IMMUNIZATION WITH AN INACTIVATED VIRUS[a]

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VARIOUS methods of vaccinating with avian encephalomyelitis virus (AEV)[1,2,4,5,6] have been reported. The risk of exposing susceptible breeders to live virus was reduced by

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using an AEV inactivated by *beta*-propiolactone (BPL) with aluminum hydroxide [$Al(OH)_3$] as an adjuvant.[2,5] Immune responses have been consistent following vaccination with such a preparation.[2] This study was undertaken to determine the longevity of the immune response to the intramuscular administration of a BPL-inactivated AEV.

MATERIALS AND METHODS

Virus. The AEV used was a 7th passage of Sumner's embryo-adapted Van Roekel strain.[7]

Source of embryos and chickens. All embryonated eggs and birds (mixed sexes) were from an AE-susceptible White Leghorn flock maintained by the Department of Animal Diseases, University of Connecticut.

Pilot studies. Tests were performed to determine concentration of BPL and times best suited for the inactivation of the virus. The concentration of various adjuvants was also tested.

Vaccine preparation. Six-day-old embryonated eggs were inoculated via the yolk sac with 0.2 ml of a 10^{-1} dilution of AEV in tryptose broth containing 1,000 units penicillin and 1,000 μg streptomycin per ml. Eight days postinoculation, whole infected embryos and allantoic fluid were harvested, pooled, and frozen at –40°C. This material was ground 5 minutes in a Waring blender while still cold, and then centrifuged 10 minutes at 1,500 rpm; the supernate was stored at –40°C until further preparation could be carried out. An aliquot of this supernate was used for potency tests.

The virus preparation was thawed, and an equal volume was added of phosphate-buffered saline (PBS), pH 7.7, containing 0.1% BPL. This mixture was then incubated 2 hours at 37°C in a water bath. An equal volume of 20% $Al(OH)_3$, containing 200 units penicillin and 200 μg streptomycin per ml, was added. An aliquot of this "finished" vaccine, pH 7.1, was removed for safety tests.

Potency tests. A titration was conducted by inoculating 0.2-ml amounts of 10^{-1} through 10^{-7} dilutions of virus, prior to inactivation, via the yolk sac of 8-day-old embryonated eggs from a susceptible flock. A titer of 10^{-5} embryo infective $doses_{50}$ (EID_{50}) per 0.2 ml was considered satisfactory.

Safety tests. Ten 1-week-old susceptible chicks were inoculated intramuscularly and 10 intracerebrally with 0.05 ml vaccine. These chicks were observed for 1 month for clinical signs of AE.

Twenty 8-day-old embryonated eggs were inoculated via the yolk sac with 0.2 ml vaccine. The eggs were candled daily to determine embryo mortality, and opened 10 days postinoculation to examine embryos for paralysis and/or muscular dystrophy.

The vaccine was considered ready for use if none of the inoculated chicks or embryos showed signs of AEV infection.

Prevaccination studies. Prevaccination serum samples were taken for the determination of neutralization indices. A chicken-infectivity titer was determined by intramuscular inoculation of 4 birds per dilution with 1.0 ml of 10^{-4} through 10^{-7} dilutions of AEV. All titers determined in these experiments were calculated according to the method of Reed and Muench.[3]

Virus neutralization tests. Virus neutralization tests were conducted according to the method of Sumner *et al.*[7]

Vaccination procedures. A total of 225 16-week-old birds were used, 100 of which served as uninoculated controls. The remaining 125 birds were divided into 5 groups, and each group was housed separately in wire-enclosed pens. The control birds were housed in the same building with the vaccinated birds. All vaccine was administered intramuscularly, and the respective groups were given 0.2, 0.4, 0.6, 0.8, and 1.0 ml of vaccine.

Challenge procedures. Four birds from each vaccinated group were challenged intramuscularly 1, 2, 3, 6, and 9 months postvaccination with at least 100 chicken infective doses (CID) of the AEV. Virus titers were determined in control birds by intramuscular inoculation at the same time intervals. Three birds from each group were bled before challenge, and pooled serums were used for the determination of virus neutralization indices. All challenged birds were housed in isolation quarters at the Department of Animal Diseases.

Criteria of infection following challenge. Birds were examined daily, and any birds showing ataxia, paralysis, or

Table 1. Results of virus neutralization tests following immunization with inactivated AEV.

Months post-vaccination	Virus neutralization doses (ml vaccine inoculated)					
	0.0	0.2	0.4	0.6	0.8	1.0
1	2	50	45	63	32	56
2	15	251	339	30	21	740
3	8	32	100	170	316	269
6	3	1,000	1,000	500	10,000	3,160
9	13	199	126	63	100	316

other signs of disease were necropsied, and representative sections were taken for histopathologic confirmation. These consisted of brain, gizzard, pancreas, proventriculus, heart, lung, spleen, liver, and muscle.

RESULTS

Results of early tests showed that a final concentration of 0.05% BPL was adequate for the inactivation of AEV when incubated in a 37°C water bath for 2 hours, as also shown by Calnek and Taylor.[2] Embryos and chicks inoculated for the safety tests showed no deaths or signs of AE infection. No undesirable reaction was observed in birds given a vaccine containing a final concentration of 10% $Al(OH)_3$.

The supernatant material obtained from centrifugation of the ground infected embryos was found to contain $10^{6.2}$ EID of AEV per ml.

Serum neutralization tests performed on prevaccination samples indicated less than 2 neutralizing doses for each bird tested.

The serum pools of the control group taken at the challenge intervals varied from 2 to 15 neutralizing doses, whereas the neutralization indices observed in vaccinated birds ranged from 21 to 10,000. Table 1 summarizes the results of the virus neutralization tests.

It was observed that all vaccinated birds were protected for 6 months, and that only 1 of 25 birds succumbed to challenge at the 9-month test period, the longest period tested. Virus titers of challenge virus in control birds varied from $10^{6.0}$ to $10^{6.6}$. Birds that received vaccine containing $10^{4.9}$ EID

Table 2. Results of challenge following immunization with inactivated AEV.

Months post-vaccination	Virus titer in controls	Positive[a]/number challenged with 1 ml 10^{-4} virus dilution				
		ml vaccine inoculated				
		0.2	0.4	0.6	0.8	1.0
1	–6.3[b]	0/4	0/4	0/4	0/4	0/4
2	–6.6	0/4	0/4	0/4	0/4	0/4
3	–6.0	0/4	0/4	0/4	0/4	0/4
6	–6.0	0/4	0/4	0/4	0/4	0/4
9	–6.0	0/5	0/6	0/3	0/4	1/7

[a] Number of birds positive for AE signs.
[b] Log base$_{10}$, minimum chicken infective dose$_{50}$ per ml.

were protected equally as well as birds that received $10^{5.6}$. These results are summarized in Table 2.

Control birds and the one vaccinated bird showed histopathologic lesions of AE. These consisted of gliosis in the cerebellar nuclei and molecular layer. Other organs appeared normal.

DISCUSSION

Immunized birds showing only 21 neutralizing doses on virus neutralization resisted challenge whereas unvaccinated birds having 15 neutralizing doses were not protected, suggesting that low neutralization indices were not necessarily an indication of AEV susceptibility. The variation observed in the number of neutralizing doses may possibly be explained by the fact that these tests were conducted with pooled samples.

Protection was afforded by 0.2 ml ($10^{4.9}$ EID) as well as by 1.0 ml ($10^{5.6}$ EID) vaccine. Therefore, the minimum amount of inactivated virus necessary to afford protection has not been determined.

Studies on the longevity of the immune response to *beta*-propiolactone-inactivated AEV have shown this to be a long-lasting phenomenon. This vaccine might be used effectively on susceptible breeder flocks and in areas where live-virus vaccination is not desired.

SUMMARY

Virus neutralization tests following immunization with *beta*-propiolactone-inactivated avian encephalomyelitis virus

showed considerable variation. Vaccinated groups of birds birds showed indices considerably higher than those obtained from the nonvaccinates.

Inactivated avian encephalomyelitis virus with aluminum hydroxide as an adjuvant protected 16-week-old chickens for at least 6 months, with only 1 of 25 birds showing signs of AE infection at the 9-month postvaccination challenge, the longest period tested.

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PROGRAM OF THE 33rd NORTHEASTERN CONFERENCE ON AVIAN DISEASES

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EXPERIMENTAL STUDIES ON THE CONTROL OF TRANSMISSIBLE ENTERITIS OF TURKEYS
R. B. Truscott
Ontario Veterinary College

PRELIMINARY REPORT ON THE DIPPING OF HATCHING EGGS WITH ANTIBIOTIC SOLUTIONS FOR THE CONTROL OF PPLO INFECTION

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Several trials were conducted to determine the effectiveness of dipping fresh chicken hatching eggs in antibiotic solutions for the control of experimental PPLO infection. Strain Hy, highly pathogenic S6 type of *M. gallisepticum* that has been maintained in birds and embryos, was used for inoculation of the fresh eggs. The amount of inoculum was 0.2 ml of dilutions from 10^{-0} to 10^{-5} of embryo material. The egg temperature was 37 C at the time of immersion and the duration was 30 minutes. The temperatures of the erythromycin solutions (400 or 800 ppm.) ranged from 4 C to 17 C. All embryos that died following an initial candling at 6 to 8 days post-inoculation were cultured for PPLO. At hatching time, re-isolations were attempted from live chicks, pipped, and nonpipped embryos. Swabbings of yolk material were used for re-isolation of the PPLO.

The criteria for infection were based on: gross pathology of embryos and chicks and various cultural tests (carbohydrate reactions, colonial formation, and hemagglutination activity).

Fewer re-isolations were obtained from the inoculated groups that had been dipped in erythromycin solution (4 C) than from the undipped-inoculated groups. The antibiotic dip solution at the higher temperatures (12 C to 16.5 C) reduced the positive isolations to a lesser degree than did the solutions at 4 C. The overall results were somewhat varied, suggesting that other uncontrolled factors may be operating.

A BRIEF DISCUSSION OF NORMAL AND ABNORMAL AVIAN BLOOD CELLS

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The accurate identification of blast cells and their early stages of development is often difficult and controversial in birds due to (1) lack of an adequate description for each cell type in each stage of its development and (2) failure to correlate equivalent cells in smear preparations and sections. In touch preparations of hematopoietic organs of the chicken, the erythroblast and thromboblast reveal nucleoli, but the granuloblast and lymphoblast do not. In sections, the erythroblast has a large conspicuous nucleolus with fairly complete margination of chromatin. A thromboblast has not yet been identified in this type of material. In sections a nucleolus is developed in the granuloblast upon its differentiation from a reticular cell and reaches its optimum size at the promyelocyte and early mesomyelocyte stages, after which it

disappears. The avian lymphoblast probably contains a nucleolus although this is not entirely established as yet.

Pathogenesis of erythroblastosis (RPL 12 strain) and myeloblastosis (Beard's strain) was followed for 14 and 20 days respectively after inoculation of the causative agents. Erythroblastosis virus produced some proliferation of the myeloid tissue in the bone marrow; the chief reaction was the intrasinusoidal erythroblasts without differentiation. Hemoglobin was absent.

The cells that proliferate in the bone marrow after injection of myeloblastosis virus do not resemble normal granuloblasts. Associated with them in the circulating blood are cells resembling lymphocytes, medium to small in size. The tumor cell and the normal reticular cell of both bone marrow and spleen are without nucleoli. There is no evidence at present that ectopic lymphoid foci are involved. In the bone marrow, the tumor involves the ground tissues between the blood sinusoids, but it is doubtful if the granulocytic series enters into the reaction. In the spleen the tumor cells are associated primarily with the ground tissues of the red pulp. It would appear as if this myeloblastic virus of high virulence had lost its neoplastic stimulation for the granulocyte series and acts now on the non-nucleolated antecedent of the granuloblast, namely the reticular cell.

SUMMARY CONCERNING AEROSOL INFECTION WITH MYCOPLASMA ALONE AND COMBINED WITH VIRUSES

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The experiments were conducted in two series. The first consisted of five trials with *M. gallisepticum* being administered by aerosol to birds ranging from 4 to 22 weeks. In a second series the B_1 strain of Newcastle disease virus (NDV) and the Dg strain of infectious bronchitis virus (IBV) as well as *M. gallisepticum* were administered to two and three week old birds. There were three groups in the second series.

Group 1. *M. gallisepticum* was given before NDV and IBV.

Group 2. All three infectious agents were given at the same time.

Group 3. *M. gallisepticum* was superimposed on the combined NDV–IBV infection after clinical symptoms had appeared.

The results were evaluated by serology, clinical symptoms, and gross pathology.

In the first series all birds except one were positive to the serological test for *M. gallisepticum* at 10 days post-infection. However, no clinical symptoms were observed and no gross lesions of respiratory diseases were present on autopsy.

In the second series no respiratory symptoms appeared until the virus infections were introduced. Upon autopsy pericardial and air sac lesion scores were the highest in the group receiving *M. gallisepticum* during the period of clinical symptoms due to NDV and IBV infections.

Mycoplasma agglutinins were detected in all birds employed in these experiments following experimental infection with the organism. Where gross lesions of respiratory disease were observed, bacteria were isolated. *Escherichia coli* was one of the most common bacterial microorganisms found.

SUMMARY OF A STUDY OF THE CHICK-EMBRYO-LETHAL ORPHAN (CELO) VIRUS BY AGAR GEL DIFFUSION TEST

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Serum samples from chickens, cows and turkeys were tested for CELO antibodies by the agar gel diffusion method as well as by serum neutralization tests in embryonating eggs and tissue culture systems. The results of the tests correlated in most of the cases. In some trials multiple bands were evident in the agar gel test. These were not observed when anti-allantoamnionic fluid (AAF) serum was added to the agar

Infective AAF was used as the antigen in the standard test. However, in limited trials tissue-culture-propagated virus and ground chorioallantoic membranes from infected embryos proved to be superior antigens.

CELO antiserum capable of neutralizing more than 200 ELD_{50} doses of the virus produced bands in 24 hours. No bands developed in 120 hours using serum samples with less neutralizing power. An anti-CELO serum sample with 2000 ELD_{50} neutralizing doses showed variation in depth, position and time of band appearance when tested against decimal dilutions of 10^{-1} to 10^{-5} of the virus.

Antiserums against the viruses of laryngotracheitis, Newcastle disease, infectious bronchitis, as well as against a virus isolated from avian lymphomatosis tissue (Fontes *et al*, Proc. Soc. Expt. Biol. Med. 97 (1958): 854.) did not show any serological relationship to the CELO virus when tested by this system.

COMMITTEE REPORT ON A TENTATIVE PROGRAM FOR THE CONTROL OF AVIAN ENCEPHALOMYELITIS

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Avian encephalomyelitis (AE) is a viral infection. Clinical manifestations are seen primarily in young chickens and are characterized by ataxia and tremor, especially of the head and neck.

Geographical distribution and hosts. The disease was first identified in chicks in New England in 1932 by Jones[9] who designated the malady as epidemic tremor. Widespread distribution of the disease in this Country as well as in many foreign countries has been confirmed.[14] The natural disease has been observed only in the chicken and pheasant but ducklings, turkey poults, young pigeons, and guinea fowl have responded to experimental infection. In view of the widespread distribution of the virus in nature and the fact that many chicken flocks become infected without clinical signs, it appears plausible that other species of birds, both domestic and feral, may encounter a similar experience. Serologic surveys for AE antibodies in various species of birds might be indicated.

Natural outbreaks of the disease may occur in chickens of all ages;

however, in young chicks (1 to 4 weeks old) the morbidity and mortality may be most pronounced. In laying birds the primary economic loss results from a decline in egg production and hatchability for 7 to 14 days.[4,14] The economic importance of the disease is of concern to the industry.

Etiology. The virus of AE can be propagated in the natural host and the chicken embryo. The virus appears to be quite stable and may remain viable for more than twenty years in a frozen state. Its infectivity and pathogenicity may be enhanced for the host upon serial passage. Strains may exhibit both neurotropic and viscerotropic properties. Further exploration of the properties of the virus should be undertaken.

Modes of transmission. The most obvious method of transmission for the AE virus is that which has been observed for many years; namely, via the egg from infected breeders to progeny. The exact mechanism of egg infection is as yet unknown. Infected eggs may either fail to hatch or produce chicks that become ataxic or paralyzed soon after hatching. Since infected breeders shed virus in the feces during the time period established for egg transmission,[4] the possibility of infection of chicks upon hatching from externally-contaminated eggs, should not be ruled out.

A less obvious situation is that which accounts for transmission from bird to bird by contact. It is known that AE virus will easily spread from egg-infected chicks to susceptible contacts either within the incubator during hatching, or during the first few days of brooding.[4] Serological tests have indicated that virus spread in growing or adult birds is equally rapid and complete. In growing birds it has been noted experimentally that infection via the intestinal tract to the exclusion of the respiratory tract can occur.[4] The reverse has not been shown to occur, but because of a paucity of information, it should not be concluded that infection via the respiratory tract also does not occur.

The disease behaves in a manner similar to other enteric-virus infection, notably polio in man. Virus is excreted in the feces in infectious quantities over a period of several days. Furthermore, oral administration of AE virus to susceptible birds produces a syndrome indistinguishable from AE as seen in field outbreaks.[4,5] Therefore, it appears that an important mode of transmission is by infection via the alimentary tract following ingestion of feces-contaminated material.

Isolation and propagation of the agent. The brain of affected chicks has been the primary source of material for virus isolation;[14] however, the feces have also been used successfully when administered orally to susceptible chicks.[4]

The source of experimental stock used for isolation studies is of prime importance, because antibodies in eggs and chicks from immune stock interfere with virus growth. The chicks and embryos from susceptible stock are best suited for virus isolation attempts.

Chicks from immune or, preferably, susceptible stock can be inoculated with 0.03 ml of 10 percent brain suspension into each hemisphere of the cerebrum. The brains should be harvested aseptically and stored frozen from any chicks showing clinical signs of AE between the 7th and 28th day post-inoculation. A pool of brains can be made for subpassage intracerebrally or orally.

The virus can be propagated in susceptible six-day-old embryonating chicken eggs by inoculation via the yolk sac with 0.2 to 0.5 ml of 10 percent brain suspension containing penicillin and streptomycin (10,000 units or μg each per ml). The brain is harvested for repassage from any embryo showing paralysis or muscular dystrophy after 10 to 12 days incubation. If none of the embryos show signs of AE infection the brains

of 4 embryos are harvested for blind passage. After 5 blind passages the pool of brains is inoculated intracerebrally or orally into susceptible chicks to determine the possible presence of a slow egg-adapting type of virus.

When attempting a primary isolation of AE virus, precautions should be used to prevent the spread of the agent. Horsfall-Bauer type isolation units are definitely desirable whenever working with field isolates in susceptible chicks.

Immunological aspect of the disease. This disease appears to be self limiting with the development of high levels of resistance; however, it is conceded that additional work is needed on this phase. Hatcherymen have for some time realized that once a breeder flock has been incriminated in the production of chicks with AE, this flock is not involved in subsequent outbreaks. The use of the embryo susceptibility test for the determination of the immunity status of flocks has confirmed the hatcherymen's observations. Approximately 60 percent of the breeding flocks tested have been immune at 6 months, whereas about 95 percent were immune at 18 months of age.[11,12] The influence of re-exposure on a continuous high level of immuniity is not known. It is also reasonably well established that chicks from immune dams carry a parental immunity for approximately 6 weeks.

Diagnosis.

1. Clinical history and signs: These characteristics are described by Van Roekel.[14]

2. Virus neutralization: The virus-neutralization test described by Sumner *et al.*[11] and Calnek and Jehnich[1] can be conducted in the usual manner using the virus dilution method and an embryo-adapted virus. The Van Roekel virus is an excellent virus to use in serologic tests because it is well adapted to embryos and has lost its ability to spread. The source of embryos for such a test is of utmost importance because the virus will not manifest its activity in embryos from an immune flock.

The embryo susceptibility test[11,12] is in essence a neutralization test for the presence of neutralizing activity contained in the yolk of eggs. The egg-adapted virus is neutralized by the antibodies in the yolk of eggs from immune stock, thereby providing a test of immunity without drawing blood samples. The virus titer is normally $10^{5.5}$ per 0.2 ml in eggs from susceptible stock. If, when the virus is titered in eggs from a flock of unknown status, the titer is $10^{3.5}$ or lower, the flock is immune. If the titer is above $10^{3.5}$ the flock is susceptible. Another method of conducting this test is to inoculate a minimum of 20 eggs each with 100 EID of virus and calculate the percentage of embryos showing signs of infection. If all embryos show signs of infection the flock is susceptible. If less than 100 percent show signs, the flock had previously experienced the virus. Recent outbreaks are generally indicated by over 50 but less than 100 percent susceptible embryos.

3. Histopathology: The lesions are described by Van Roekel.[14]

Control of the disease. For the control of avian encephalomyelitis, two general approaches should be considered. One takes advantage of the knowledge that the disease is extremely widespread and that it is the occasional flock in this country that goes through its lifetime without encountering the natural disease.[11,12] By hatching only from those flocks known to be immune, losses from the disease could be controlled. It should be pointed out, however, that only losses and not the disease itself would be controlled, and that such a program is advantageous only for the hatchery with enough supply flocks to select.

The other and presumably the more advantageous method for control is immunization. It has been ably demonstrated that vaccination will

afford a lasting immunity against the disease.[10] Several methods have been developed and each will be discussed.

1. *Wing-web or intramuscular-live virus*: The chief advantages to the use of wing-web vaccine are immunogenic capability[8,10] and knowledge that all birds within a flock are exposed. Such vaccines have been used by several hatcheries on a large scale with good success. Vaccination is during the growing period. There are two major disadvantages. In addition to the necessity of handling each bird, experimental data indicate that a rather severe postvaccination reaction can sometimes occur.[2,8]

2. *Beta-propiolactone-inactivated vaccine*: This vaccine, although also requiring the handling of individual birds, does not elicit any postvaccination reaction. The inactivation procedure leaves antigenicity relatively unaltered and provides an immunogenic vaccine.[2,7,9] Because a much higher virus dosage per bird is required, the cost of this vaccine greatly exceeds that of live virus vaccines.

3. *Live virus for oral administration*: Oral vaccination[5] requires a virus strain that can be maintained in a state closely approximating that of natural virus. A chief disadvantage to the vaccine is that of production since this virus does not produce visible lesions in susceptible embryos. Virus assay and titration are necessarily done by oral inoculation of susceptible chicks. When given by the oral route to susceptible birds over three weeks of age, it is highly immunogenic, yet induces no clinical signs. The vaccine can be administered either via the drinking water or *per os* by inoculating a few birds (1 percent or less) per pen. In the latter case, it easily spreads to contacts. No differences between this virus and that found in field outbreaks have been distinguished. Also, the vaccine-induced infection is not discernible from natural infections.

A number of points should be considered regarding the use of live versus inactivated virus for the control of AE: a. Additional infection added to the poultry population by use of live virus. b. Possibility of spread from vaccinated flocks to neighboring susceptible birds. c. Feasibility of control by eradication using killed virus. d. Problem of immunizing flocks already in production.

The widespread occurrence of the disease tends to invalidate concern over the use of live virus. The fact that vaccination of breeder replacements would involve only a very small percentage of the total poultry population would make virtually impossible an attempt to eradicate the disease by the use of killed vaccine. Since almost all flocks become infected eventually, the use of live virus vaccines would not produce any more foci of infection than would normally exist, but rather would time the infections advantageously.

In the opinion of the majority of this committee the most logical approach, would be to use a live virus vaccine administered orally to breeder replacements between 8 and 20 weeks of age. Parental immunity can interfere with immunization in chicks prior to 8 weeks of age.[5,7] This, in conjunction with the use of inactivated vaccine for susceptible flocks already in production, or in areas (or countries) where the disease is not enzootic should make a great stride forward toward the control of AE.

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A BRIEF REPORT ON ANATOMY, HISTOLOGY, AND REACTIVITY OF AIR SACS IN THE FOWL

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The number of air sacs in birds is variable. The chicken as an embryo potentially has 6 pairs: cervicals, 2 pairs of interclaviculars, anterior thoracics, posterior thoracics, and abdominals. The 4 interclaviculars are fused into one sac. This reduces the number to a total of 9 in ducks; in the chicken the cervicals are also fused across the mid-line, reducing the total to 8, 3 pairs and 2 singles; and in turkeys, the cervicals are not fused but one pair of thoracics is absent, making a total of 7.

Diverticula arise from the cervicals, interclavicular, and abdominals; they are absent from both thoracics. In general, diverticula form air cushions around the moveable joints. Also they send extensions into adjacent bones, such as neck vertebrae, humerus, pectoral girdle, anterior

ribs, most thoracics, lumbar and sacral vertebrae, and some parts of the pelvic bones.

The abdominal air sac membrane is composed of two epithelial layers with collagenic and elastic fibers between. Reticular fibers are absent. The flattened cells on the endodermal side are smaller than those on the peritoneal side. In the thoracic air sacs the endodermal cells may be ciliated and include goblet cells. This condition is atypical for the abdominal air sacs. The abdominal air sacs are vascularized only in part. Nerves are distributed to the blood vessels and are absent from the avascular parts of the abdominal sacs. Among the connective tissues are tissue mast cells, fibroblasts, and smaller cells that are probably histocytes.

When the air sac is stimulated, the squamous cells of the abdominal sac multiply, become more compressed laterally, and eventually attain columnar conditions, with ciliated borders and interspersed goblet cells. Also, heterophils leave the vascular channels and enter the tissues. Macrophages develop and ingest foreign particles. Sometimes stimulation causes a thickening of the endodermal epithelium and a stratified non-ciliated epithelium is developed.

REPORT ON THE PULLORUM DISEASE TESTING RESULTS PRESENTED AT THE 33RD MEETING OF THE NORTHEASTERN CONFERENCE ON AVIAN DISEASES

A summary of pullorum testing data was collected and compiled for 1960–61 by a special committtee of the Conference (W. R. Teeter, Chairman, F. W. Rea, and H. Van Roekel). Fourteen states and two Canadian provinces cooperated in furnishing the testing data. The testing results concerning chickens and turkeys are listed in the table. No reactors were detected among tested chicken breeding flocks in Maine, New Hampshire, Nova Scotia, Rhode Island, Vermont, and West Virginia. Seven states and one province reported no pullorum "breaks" among previously nonreacting flocks. A pullorum infection was detected among turkey breeding flocks in three states. Pullorum break was detected in only one state.

In addition to the chickens and turkeys tested, the following were tested: 595 bantams, 3,106 ducks, 331 game birds, 105 geese, 1,011 grouse, 847 guinea fowl, 505 pea fowl, 20,480 pheasants, 6 pigeons, 1,632 quail, 5 swan, and 6 miscellaneous birds. No reactors were detected among fowl other than chickens or turkeys.

Special credit should be extended to Dr. Teeter and his co-workers for summarizing the data submitted.

H. Van Roekel, Permanent Secretary

Summary of Pullorum Disease Testing Data
Reported by Northeastern Conference on Avian Diseases 1960–61

	1	2	3	4	5	6	7
Conn.	80*	457,280	0.0004	78	0	71	456,530
	13**	8,432	0.00002	12	0	13	8,403
Del.	248	523,135	0.0036	242	6	244	507,369
	4	1,862	0.0	4	0	4	1,862
Maine	175	1,807,602	0.0	172	0	172	1,796,705
	1	154	0.0	1	0	1	154
Md.	331	247,407	0.008	315	1	330	247,244
	9	7,560	0.0	9	0	9	7,560
Mass.	176	675,240	0.0034	169	2	95	504,210
	19	20,894	0.0	19	0	10	13,043
N. C.	1,534	3,135,072	0.001	1,485	7	1,499	3,105,440
	81	134,974	0.02	72	1	79	125,749
N. H.	134	609,858	0.0	130	0	130	588,067
	10	14,663	0.0	10	0	10	14,663
N. J.	144	398,123	0.0065	139	5	141	392,937
	10	9,373	0.0	10	0	10	9,210
N. Y.	117	504,176	0.0038	98	0	98	460,289
	21	24,487	0.0	21	0	21	24,487
Nova Scotia	20	59,507	0.0	20	0	20	59,507
	3	338	0.0	3	–	3	338
Ontario	2,130	1,295,985	0.04	2,122	–	2,085	1,268,961
	133	127,382	0.0	133	–	133	127,382
Pa.	824	1,453,846	0.02	823	4	818	1,437,386
	47	21,998	0.02	45	0	45	21,414
R. I.	32	63,415	0.0	26	0	26	61,415
	2	666	0.0	2	0	2	666
Vt.	84	134,131	0.0	84	0	84	134,131
	4	1,023	0.0	4	0	4	1,023
Va.	876	1,135,421	0.0268	780	15	870	1,116,446
	282	203,868	0.0	282	0	282	203,868
W. Va.	331	89,143	0.0	331	0	331	89,143
	22	12,758	0.0	22	0	22	12,758
TOTAL	7,236	12,589,341	0.013	7,014	40	7,014	12,225,780
	661	590,432	0.011	649	1	648	572,580

1=Number of flocks tested.
2=Number of birds tested.
3=Percent positive (based on number of birds tested).
4=Number of flocks 100% tested and with no reactors.
5=Number of breaks in flocks 100% tested and with no reactors the previous season.
6=Number of pullorum-clean flocks.
7=Number of pullorum-clean birds.
*=These figures under each state represent chickens.
**=These figures under each state represent turkeys.

REPORT OF THE COMMITTEE ON NOMENCLATURE AND REPORTING OF DISEASES, NORTHEASTERN CONFERENCE ON AVIAN DISEASES, JUNE 1961

This report presents information on the incidence of disease in the area encompassed by this Conference. It is hoped that a nomenclature and system of reporting of diseases can be developed that may serve to standardize the reports from the various laboratories.

The statistics are based on the calendar year 1960 except those for Ontario and Delaware, which are based on a fiscal year. Each unit statistic represents a single case of consignment to the laboratory, and therefore approximates an outbreak of disease on a premises. The figures are reported by state rather than by member laboratory.

The incidence of disease is reported by age groups for chickens that coincide roughly with the brooding, growing, and adult life of the birds. A separate category indicates incidence of disease in species other than chickens. Whenever possible the identity of the species is indicated. T-turkey; Ph-pheasant; D-duck; Pgn-pigeon; Qu-quail; Gu-guinea; Can-canary; Ptd-partridge; Gse-geese; Hk-hawks. More than one species were combined and designated by letter O.

All infectious and contagious, parasitic and nutritional diseases plus such miscellaneous and unclassified conditions and diseases as may be of significance or interest are reported. However, limitations of space and variations in systems of reporting by the various laboratories make it necessary to be somewhat abitrary in preparing the report, and some discrepancies and omissions are unavoidable.

Specific nomenclature is used wherever possible to indicate specific disease entities. However, for expediency some classifications are used in a generic sense and singleness or identity of etiology is not indicated, Ascaridiasis, coccidiosis, and pox are examples. Nutritional diseases are classified according to clinical syndrome. Thus, under the term rickets are included cases of improper calcification of bones whether due to avitaminosis-D, a deficiency or imbalance of minerals as calcium or phosphorus, or an excess of sulfur in the diet. The statistics are presumed to be essentially accurate even though not all diagnoses are based upon the isolation or identification of the etiological agent.

Certain unavoidable discrepancies are indicated by letter, which also appears by the statistic to which it applies.

[a]—Figure includes all avian species.
[b]—Figure includes all chickens regardless of age.
[c]—Figure includes all worms.
[d]—Figure includes unidentified forms of leukosis.
[e]—Figure includes all forms of leukosis.

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Summary of Poultry Diseases
Reported by Northeastern Conference on Avian Diseases
1960

State		Conn.	Del.	Maine	Md.	Mass.	N.H.	N.J.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Ontario
Consignment totals		1309	2728	1068	1423	1468	1664	5728	5524	4810	6016	791	240	3194	468	4871
Acariasis	0-4 wk				2			a 54			b 37					
	1-5 mo				24	1			3			4			1	
	+ 5 mo	5		1	29	8	3		73			21				1
	Other				Pgn-1				0-3		0-3					
Anatipestifer infection	Other								D-284							
Aneurysm, dissecting	Other					T-7			T-5	T-12	T-18				T-1	T-35
Ascaridiasis	0-4 wk		4		1			a791	1	14	b 565		b12	28	c 1	1
	1-5 mo	3	95	6	1	1	5		98	344		4		98	c 22	56
	+ 5 mo	37	30	60	2	13	22		286	212		4		68	c 16	196
	Other		0-4	Ptd-1		0-3			0-8	0-15	0-18			T-16	cT-9	0-12
Aspergillosis	0-4 wk		53	2	43			a 13	8	10	b 68				6	2
	1-5 mo		13						18	8					1	5
	+ 5 mo		1	2		3	1		13	1						6
	Other		T-12	T-1	0-3	T-3	T-1		0-41	T-8	0-41				T-1	0-35
Avitaminosis A	0-4 wk		2	1	12			a 38		3	b 6			2		
	1-5 mo		2		3					5				5		5
	+ 5 mo								6	1				3		13
	Other				D-2				0-2	0-1						
Blue comb complex	0-4 wk							a 65			b 137		b 2	4	3	1
	1-5 mo	1	5		11					13				21	1	2
	+ 5 mo	11	3		10		4		19	41				40	2	20
	Other	T-4	T-6		Gse-2	T-9	T-1		0-13	T-27	0-61			T-197	T-28	T-111
Botulism (limberneck)	0-4 wk		1					a 26			b 17					
	1-5 mo		4		16				12	9				3		
	+ 5 mo				1				2	5		2		1		
	Other		D-1		0-15	0-2			D-2	0-6	0-10					
Bronchitis, infectious	0-4 wk	1	14	3				a 17	3	17	b 80	1		1		9
	1-5 mo	9	43	7	2	13	2		13	54		1		5	1	16
	+ 5 mo	2	3	2	7	14	1		13	10		5				4

State		Conn.	Del.	Maine	Md.	Mass.	N.H.	N.J.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Ontario
Consignment totals		1309	2728	1068	1423	1468	1664	5728	5524	4810	6016	791	240	3194	468	4871
Capillariasis	0-4 wk							a 574		1	b 248		b11			
	1-5 mo	3	3	8	4	3	9		15	63		6		2		10
	+ 5 mo	105	5	94	8	89	101		200	140		5		4		44
	Other	T-3				0-3	T-1		0-3	0-1	0-19					0-7
Cholera, fowl	1-5 mo		15		10			a 81	3	30	b 58		b 3	7	1	1
	+ 5 mo	8	10	8	5	11	1		36	55					1	27
	Other	T-7			0-4	0-6			0-93	T-24	0-20			T-60	T-6	0-27
Chronic respiratory disease & infectious sinusitis	0-4 wk	1	112	13	9	3	18	a 380	26	66	b 624	3	b 7	40		38
	1-5 mo	109	928	103	58	101	90		414	648		21		189	13	297
	+ 5 mo	40	21	19	11	76	18		147	18		4		41		151
	Other	T-8	T-34		T-12	T-21	T-6		0-33	T-20	0-76		T-1	0-181		0-156
Coccidiosis	0-4 wk		64	5	26	7	26	a1636	37	112	b 797		b25	45	6	25
	1-5 mo	69	608	44	180	76	49		551	709		21		190	56	328
	+ 5 mo	19	16	29	51	30	1		224	110		7		32	8	191
	Other	Ph-2	T-10		0-12	0-28	T-4		0-37	0-55	0-34			T-82	T-24	0-88
Coryza, infectious	0-4 wk							a 7		2	b 15		b 1			1
	1-5 mo	1			3					15						4
	+ 5 mo		1		4		1			14						8
Encephalomalacia	0-4 wk	19	1	4	10	3				10	b 19	1				
	1-5 mo		3			2	1		3	5		3			1	
	+ 5 mo				2											
	Other	T-1								0-1	T-1					
Encephalomyelitis, avian	0-4 wk	44	70	12	24	36	23	a 33	64	79	b 129	7	b14	16	4	90
	1-5 mo	3	3		1	6	4		1	5		3		1	1	22
	+ 5 mo	1							2					1		
Enteritis unidentified	0-4 wk		25	2	15		2	a 270	2		b 207			23	3	27
	1-5 mo		176	9	69		19		51			2		120	5	54
	+ 5 mo		19	34	24	5	26		26			80		69	4	72
	Other		0-27		0-11	0-7	T-2		0-74		0-16			T-197	T-5	0-70
Enteritis, ulcerative	0-4 wk							a 3	1		b 30					
	1-5 mo	3	1		6	11			61						1	2
	+ 5 mo	1			7				3							
	Other	0-4			0-6	0-6			0-12	Qu-12	0-5					T-1
Erysipelas	+ 5 mo				2			a 4	4		b 4					1
	Other	T-1		T-1	T-2	T-9	T-3		0-10	T-16	T-17	T-1	T-2		T-2	T-19

State		Conn.	Del.	Maine	Md.	Mass.	N.H.	N.J.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Ontario
Consignment totals		1309	2728	1068	1423	1468	1664	5728	5524	4810	6016	791	240	3194	468	4871
Hemorrhagic syndrome	0-4 wk		7		4			a 341	3	2	b 182			11		3
	1-5 mo	15	84		32	2	6		91	18				111	4	29
	+ 5 mo		1		1		2		7					15		2
	Other				T-4					T-8	T-1			T-13		T-5
Hepatitis, duck virus	Other								D-69		D-1					
Hepatitis, infectious avian (vibrionic)	0-4 wk							a 18		1	b 44					
	1-5 mo	3			1	4	1		10	28				2		3
	+ 5 mo	52	1			34	15		173	83				1		8
Hepatitis, turkey virus	Other	T-1			T-2	T-33				T-1	T-6					
Hepatitis unidentified	1-5 mo							a 77	11		b 35					3
	+ 5 mo			6	2	1	6		37						1	69
	Other				Ph-2	0-4			0-2		T-4					0-4
Hexamitiasis	Other					T-2			Ph-1	T-1	0-7					
Histomoniasis	0-4 wk						1	a 342	1	1	b 216		b 1	1		2
	1-5 mo	8	8	3	10	14	19		106	100				24		45
	+ 5 mo	5	3		4	2	11		34	83		2		5		18
	Other	T-2	0-11		0-6	T-20	T-3		0-36	T-52	0-56	T-4		T-67	T-13	0-79
Laryngotracheitis, infectious	0-4 wk	1	13		8			a 81			b 73					1
	1-5 mo	19	148	32	67	5			3	1		4		9		8
	+ 5 mo	12	4	35	5	16			2	6		6		1		8
	Other								Ph-1							0-2
Leucocytozoon infection										T-2						
L Erythroblastic)	1-5 mo				d 68				3		b 6			1		1
E Myeloblastic)	+ 5 mo	2			d 41	2			6			12		1		1
U Neural	0-4 wk		1				e 2	ae	2		b 442		b17			
K	1-5 mo	32	127	26	5	90	e150	1411	282	169		11		55	2	224
O	+ 5 mo	20	21	19	5	33	e230		193	29		10		39	1	95
S	Other													T-1		0-13
I Ocular	1-5 mo		6	3	1				11	13	b 79		b 2	4	1	2
S	+ 5 mo		8	2		7			117	18				5		43
Osteopetrosis	0-4 wk									5	b 22					
C	1-5 mo		5			2			4	19				2		
O	+ 5 mo	2		1		2			34	2				3		1
M	Other															T-1
P Visceral	0-4 wk		5							2	b 839	1	b30	6		
L	1-5 mo	39	248	32	41	59			179	300		8		79	3	93
E	+ 5 mo	63	47	110	46	154			638	224		203		117	2	403
X	Other		T-6		T-2	T-8			T-6	T-3	0-8			T-2		0-8

State		Conn.	Del.	Maine	Md.	Mass.	N.H.	N.J.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Ontario
Consignment totals		1309	2728	1068	1423	1468	1664	5728	5524	4810	6016	791	240	3194	468	4871
Listeriosis, avian											b 6					
Newcastle disease	0-4 wk	2	31		17		1	a 127	2	3	b 41	2	b 1			
	1-5 mo	17	63	4	36		7		33	14		3			1	
	+ 5 mo	26	7		2	3	4		17	2		11				
	Other										0-2					
Omphalitis	0-4 wk	11	42	28	13	7	104	a 49	34	161	b 56	6	b 2	5	23	17
	1-5 mo	2	2						3	4				2		
	Other		T-2		T-2	T-1	T-4		0-9	T-13	0-11				T-2	T-14
Ornithosis & psittacosis								0-2	Can-1	T-1	0-18					0-1
Paracolon infection	0-4 wk				1									5		
	1-5 mo				3					1				3		1
	+ 5 mo	1	1	1						3						5
	Other				T-1		T-1			T-1				T-5		0-4
Paratyphoid infection	0-4 wk		4		3	3	1	a 13	6	27	b 1				2	10
	1-5 mo					2			1	42						5
	+ 5 mo	7			2	3			3	32						2
	Other	T-2	T-1	T-1	T-2	0-19			0-66	T-1	0-12					0-16
Pediculosis	1-5 mo		3			1		a 217	4		b 147		b 2	1	1	
	+ 5 mo		6		8	20			75					13	1	15
	Other		PF-1			0-2			0-11		0-6					0-1
Peritonitis	0-4 wk			2			1	a 313			b 127		b 1			2
	1-5 mo		1	6	2	1	2		13	5				3		2
	+ 5 mo	3	8	20	7	5			341	61				8		72
	Other				T-1	T-1			0-39	0-7	0-3			T-1		T-3
Perosis	0-4 wk				2		3	a 38	4	2	b 30				3	2
	1-5 mo				2	3	5		30	6				3		9
	+ 5 mo								5					1		
	Other				D-1				0-10		T-8			T-3		0-7
Pox	0-4 wk						3	a 35	1		b 47					
	1-5 mo		24		4	1			5	8				4		1
	+ 5 mo	2	9	1	4	6	1		28	8				10	1	35
	Other					0-2			0-3		0-4			T-1		0-5
Pullorum disease	0-4 wk		8		17			a 15	1	39	b 24	1	b 2	4	2	3
	1-5 mo		2	1	2				4	8				1		8
	+ 5 mo	1			16	6			5	13				2		2
	Other		Gu-1		T-1					0-1	T-1					

State		Conn.	Del.	Maine	Md.	Mass.	N.H.	N.J.	N.Y.	N.C.	Penn.	R.I.	Vt.	Va.	W.Va.	Ontario
Consignment totals		1309	2728	1068	1423	1468	1664	5728	5524	4810	6016	791	240	3194	468	4871
Respiratory infection (unidentified)	0-4 wk						28	a 369	2	1	b 27			3	7	
	1-5 mo					3	18		42	7				6	81	
	+ 5 mo					8	3		20	7				8	2	
	Other					Pgn-1			0-25					T-2	T-7	
Riboflavin deficiency	0-4 wk	3	5			2		a 11	2	5	b 12			3		2
	1-5 mo						2			2				2		
	Other														T-1	
Rickets & osteomalacia	0-4 wk		16	5		1		a 16	4	7	b 25		b 1	8		
	1-5 mo	2	15	4		5			6	15		8		3	2	
	+ 5 mo					3			35	3				2		
	Other								0-7	T-4	T-4			T-8		
Synovitis, infectious	0-4 wk		2	1	10			a 65	6	23	b 114		b 2	1		1
	1-5 mo	25	116	34	72	16	55		180	193		13		29	22	82
	+ 5 mo	22	3	14	15	10	31		45	18		5		5	2	22
	Other	T-1			T-7	T-5			T-6	T-26	T-16		T-1	T-52	T-6	0-52
Tendon, ruptured (gastrocnemius)	1-5 mo	1		2	1	5	4		4		b 21	3				5
	+ 5 mo	2		1		9	11		4	1						1
	Other															T-1
Teniasis	0-4 wk							a 252		3	b 138			5		
	1-5 mo		8			2	3		3	24				15		2
	+ 5 mo	2	3	3		8	10		18	56		1		15	2	13
	Other								0-2	T-1	0-6					
Trichomoniasis	1-5 mo				1				1	8						2
	+ 5 mo				5		1			9						1
	Other		T-3					Pgn-4	0-3	T-5	0-4			T-1		0-5
Tuberculosis, avian	1-5 mo							a 1			b 5					1
	+ 5 mo		1		8				4	1				4	1	38
	Other	Hk-1							0-2		Ph-1					
Tumors	1-5 mo	4	5	7	8		7	a 190	23		b 55		b 1			2
	+ 5 mo		2	8	15		7		180						1	4
	Other					0-4			0-6		0-3			T-1		0-3
Typhoid, fowl	0-4 wk							a 13		2	b 16					
	1-5 mo		1		1					18						
	+ 5 mo		2		2	1				31				3		2
	Other		T-1							T-2				T-2		
Xanthomatosis	1-5 mo							a 6	2		b 5					
	+ 5 mo								4							2

Code: a - figure includes all avian species
b - figure includes all chickens regardless of age
c - figure includes all worms

Avian Diseases

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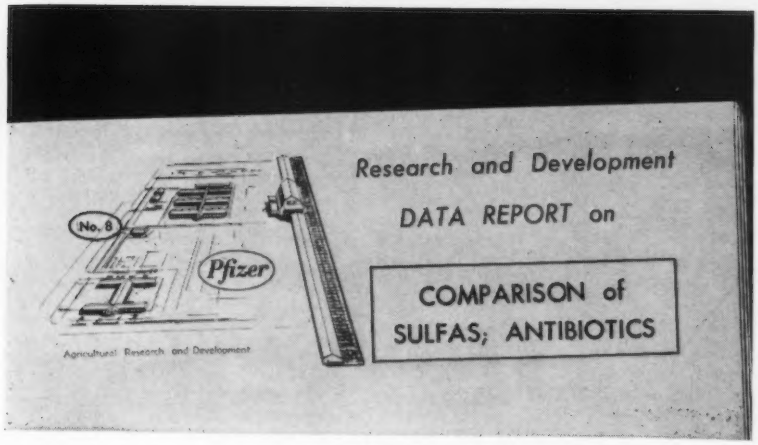

Studies on control of fowl cholera

This new report, Technical Bulletin 23, comes from the Veterinary Department of South Dakota State College. It compiles all the experimental data on fowl cholera since work was undertaken at that station in 1944. Latest findings in symptomology and pathology are investigated and explained. Control measures, sulfonamide drugs and antibiotics, are compared as to their effectiveness in preventing losses from the disease.

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1. Johnson, C.: Paper presented at the 45th Annual Meeting of the Poultry Science Association, North Carolina State College, Raleigh, North Carolina, Aug. 7-10, 1956.

2. Shumard, R. F.: Paper presented at the First National Symposium on Nitrofurans in Agriculture, Michigan State University, East Lansing, Michigan, Sept. 28-29, 1956.

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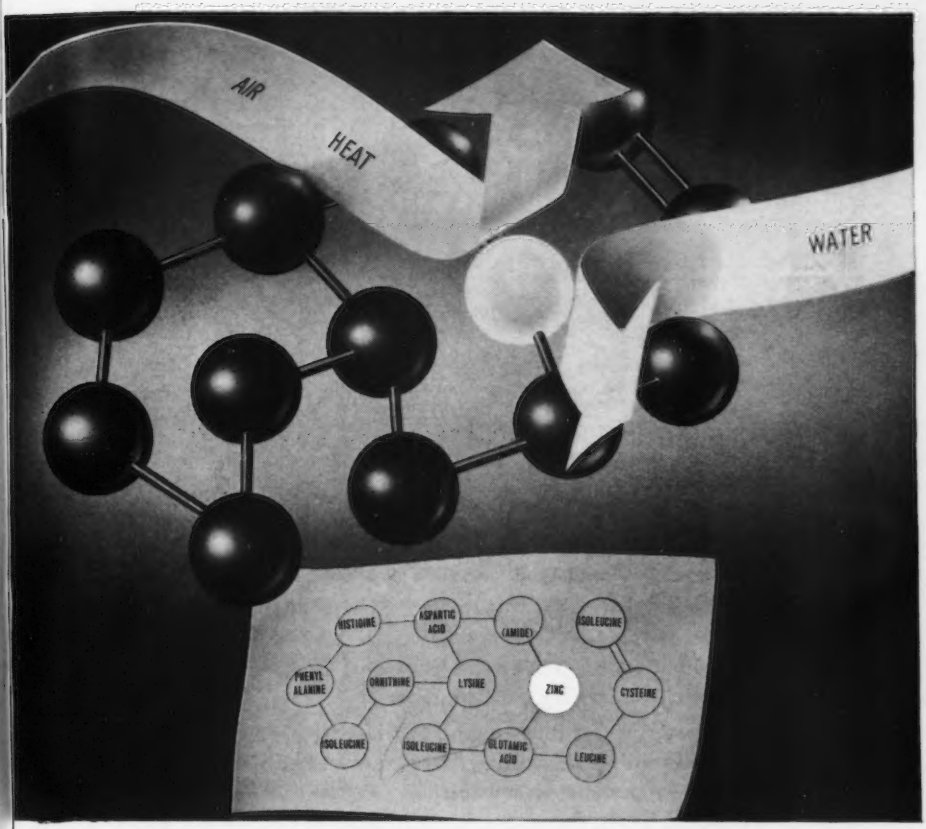

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References: 1. Plummer, A., Gaunt, R., and Bellotf, G.: CIBA Research Laboratories. 2. Couch, J. R.: First Conference on the Use of the Tranquilizing and Antihypertensive Agent SERPASIL in Animal and Poultry Production, College of Agriculture, Rutgers, The State University, New Brunswick, N. J., May 7, 1959. 3. Weiss, H. S.: Ibid. 4. Burger, R. E.: Ibid. 5. Gilbreath, J. C.: Ibid. 6. Parker, E. L.: Second Conference on the Use of Reserpine in Poultry Production, The Institute of Agriculture, University of Minnesota, St. Paul, Minn., May 6, 1960. 7. Ringer, R. K.: Ibid. 8. Waibel, P. E.: Ibid. 9. Morrison, W. D.: Ibid. 10. Carlson, C. W.: First Conference, etc.

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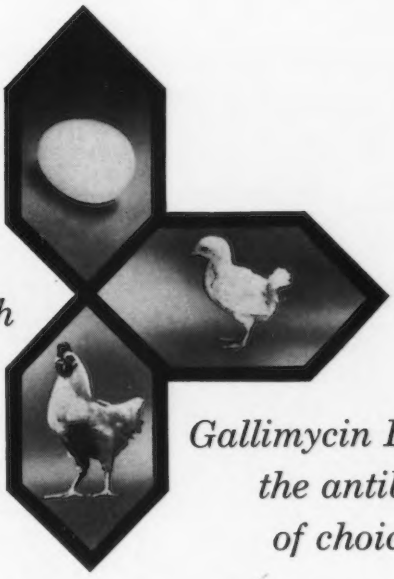

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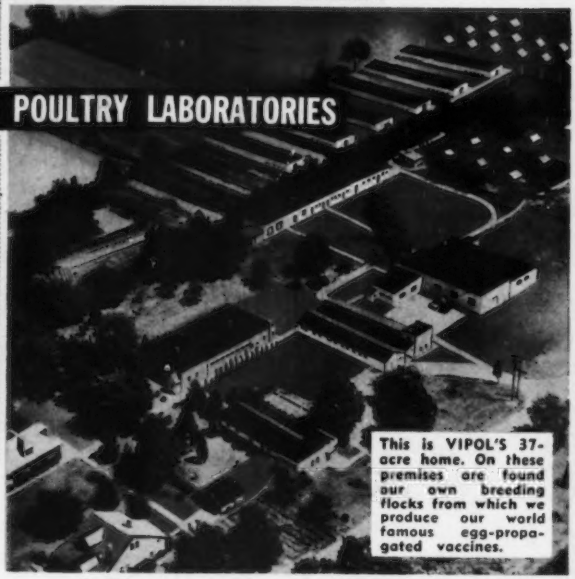

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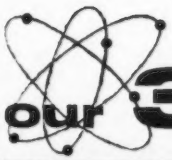

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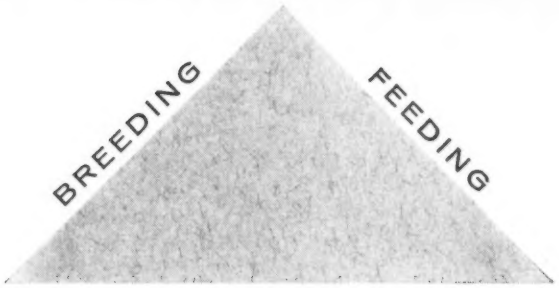

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